Yashoda Shikshan Prasarak Mandal's

YASHODA TECHNICAL CAMPUS, SATARA

NH-4, Wadhe Phata, Satara. Tele Fax- 02162-271238/39/40

Website- www.yes.edu.in, Email-registrar_ytc@yes.edu.in

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Affiliated to DBATU Lonere & Shivaji University, Kolhapur/ MSBTE, Mumbai.

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Institute Code – 6757

Prof. Dasharath Sagare	**Prof. Ajinkya Sagare**	**Dr. Vivekkumar Redasani**
Founder, President	**Vice-President**	**Director**

NAAC SSR II CYCLE

Criterion III

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Prof. Dasharath Sagare	Prof. Ajinkya Sagare	Dr. Vivekkumar Redasani
Founder, President	Vice-President	Director

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QM No.	QM NAME	Page No.
3.3 Research Publications and Awards		
3.3.1	Number of research papers published per teacher in the Journals as notified on UGC CARE list during the last five year	1-1455

Number of books and chapters in edited volumes/books published and papers published in national/ international conference proceedings per teacher during last five years

Year	2022-23	2021-22	2020-21	2019-20	2018-19	Total
Number	89	41	36	14	15	195

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Sr. No.	Particulars	Number of Publications	Supportive Documents
1	Research Publications: 2022-23	89	1-617
2	Research Publications: 2021-22	41	618-962
3	Research Publications: 2020-21	36	963-1227
4	Research Publications: 2019-20	14	1228-1316
5	Research Publications: 2018-19	15	1317-1455
Total		195	

Criterion III: - Research, Innovations and Extension

Sr. No.	Title of paper	Name of the author/s	Name of journal	Link to article / paper / abstract of the article
1	Dettol Brand Efforts during Covid-19	Dr.R. R. Chavan	International Journal of Marketing and Technology	https://www.ijmra.us/project%20doc/2022/IJMT_SEPTEMBER2022/IJMT1Sep22.pdf
2	A Study of Online Buying Behavior of Consumers toward Standardized Products	Dr. S. A. Bhosale	Sanskruti International Multidisciplinary Research Journal	http://simrj.org.in/SpecialIssues.aspx
3	Buyer's perception of e-vehicle in satara	Dr.R. R. Chavan and Shubham Siddeshwar Jadhav	International Journal of Current Research	DOI: https://doi.org/10.24941/ijcr.44627.01.2023
4	Study of Factors Affecting the National Anonymously:Dark Web	Prof.Pranjali S.Gade	IJRASET	https://www.ijraset.com/best-journal/the-dark-web-privacy-and-anonymity
5	Oral cancer Detection Using Image processing and Deep Neural Networks	Asst.Prof.S.V.Thorat	IRJET	https://www.irjet.net/archives/V9/i12/IRJET-V9I12130.pdf
6	Secure Desktop Computing in the Cloud	Asst.Prof.S.V.Thorat	IRJET	https://www.irjet.net/archives/V10/i1/IRJET-V10I128.pdf

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7	Secure Cloud Computing	Dr.Prof.S.P.Jadhav and Prof. S.S, jadhav	IRJET	https://www.irjet.net/archives/V10/i1/IRJET-V10I128.pdf
8	An overview of Bluetooth Technology and it's communication application	Prof.Vanmala V.Kadam	IJRASET	https://www.ijraset.com/best-journal/bluetooth-chat-an-android-chatting-app-based-on-bluetooth
9	Letest Cyber Security Trends	Prof.Pranjali S.Gade	IRJET	https://www.irjet.net/archives/V9/i12/IRJET-V9I12196.pdf
10	An overview of Bluetooth Technology and it's communication application	Prof.Pranjali S.Gade	IJRASET	https://www.ijraset.com/best-journal/bluetooth-chat-an-android-chatting-app-based-on-bluetooth
11	Security Factors Affecting Internet of Things	Prof.Vanmala V.Kadam	IJRASET	https://www.ijraset.com/best-journal/security-factors-affecting-internet-of-things
12	The Impact of Stock market on Indian Economy	Dr.Prof.S.P.Jadhav and Prof. S.S, jadhav	IJRASET	https://www.ijraset.com/best-journal/security-factors-affecting-internet-of-things
13	The ERP system implementation and current trends in ERP	Dr.Prof.S.P.Jadhav Asst.Prof.S.V.Thorat	IJSER	NA
14	Web 3.0- Future of the Internet	Asst.Prof.S.V.Thorat	IRJET	https://www.irjet.net/archives/V10/i1/IRJET-

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15	Robotics:Social Robot	Prof.Vanmala V.Kadam and Prof.S.S.Jadhav	IJRASET	https://www.ijraset.com/best-journal/robotics-social-robot
16	Virtual Smart Phones	Dr.Prof.S.P.Jadhav	IRJET	https://www.irjet.net/archives/V8/i7/IRJET-V8I7384.pdf
17	Overview of Social Media	Prof.S.S.Jadhav	IJRASET	https://www.ijraset.com/best-journal/overview-of-social-media
18	Blue Brain Technology	Prof.Vanmala V.Kadam	IJRASET	https://www.ijraset.com/best-journal/blue-brain-technology
19	Embedded System- based Intelligent Wheelchairs for Disabled People	Prof.Vanmala V.Kadam	IRJET	https://www.irjet.net/archives/V9/i12/IRJET-V9I12100.pdf
20	Mobility Operation in the 5G Network between Colorful Access Networks	Prof.Vanmala V.Kadam and Prof.S.S.Jadhav	IRJET	https://www.irjet.net/archives/V10/i1/IRJET-V10I141.pdf
21	API Testing Using Postman	Prof.S.S.Jadhav	IJRASET	https://www.ijraset.com/best-journal/api-testing-using-postman-tool#:~:text=Postman%20is%20an%20API%20client,more%20efficient%20and%20less%20tiresome.
22	IOT:What is IOT and its Adavantages	Prof.Shweta Thorat	IJRASET	https://www.ijraset.com/best-journal/a-

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24	Secure Desktop Computing in the Cloud	Asst.Dr.S.P.Jadhav	IRJET	https://www.irjet.net/archives/V10/i1/IRJET-V10I128.pdf
25	Green Computing for Internet Of Things	Harshal Gajanan Patil, Rasika Vishnu Tapase, Asst.prof.P.S.Gade, Asst.prof.V.V.Kadam	IRJET	https://www.irjet.net/archives/V9/i12/IRJET-V9I12131.pdf
26	Dairy Farm	Pratiksha Mahadik, Mansi Bhandari, Prof. Snehal Jadhav	IJRASET	https://www.ijraset.com/research-paper/dairy-farm
27	WI-FI Technology	Prof.Shweta Thorat	IJRASET	https://www.ijraset.com/best-journal/wi-fi-technology
28	Research on Esterification Reaction Under, Microwave Assisted Synthesis Of Butyl Benzoate For Green Chemistry	A. A. Jadhav, R. P. Devale, K. C. Jagtap, S. D. Patil	International Journal of Scientific Development and Research	https://www.ijsdr.org/papers/IJSDR2206017.pdf
29	Review on General Purpose of Catalysis In Green Chemistry	S.M. Pawar, R. P. Devale	International J. of Creative Research Thought	https://ijcrt.org/papers/IJCRT22A6527.pdf
30	Influence of Newly Synthesized Superdisintegrant on Dissolution Rate Enhancement of Carbamazepine using Liquisolid Compact Technique	V. G. Raut, B. P. Chaudhari, V. K. Redasani	Asian Journal of Research in Pharmaceutical Sciences	https://ajpsonline.com/AbstractView.aspx?PID=2022-12-2-4

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32	Degradation Study of Different Brands of Antipyretic Tablets by UV Spectroscopy	U. Rangat, Anjan Ladage, B.P. Chaudhari , V.K. Redasani	World Journal of Pharmacy and Pharmaceutical Sciences	https://storage.googleapis.com/journal-uploads/wjpps/article_issue/1661393212.pdf
33	Cleaning Validation of Tablet Compression Machine By Swab Sampling	P. D. Khalate, P. S. Londhe, B.P. Chaudhari, V.K. Redasani	World Journal of Pharmaceutical Research	https://wjpr.net/archive_show/2022/VOLUME%2011,%20AUGUST%20ISSUE%2011
34	Quality by Design (QbD) concept Review in Pharmaceuticals	Kaustubh Jagtap, B.P. Chaudhari , V.K. Redasani	Asian J. Research Chem.	https://www.ajrconline.org/AbstractView.aspx?PID=2022-15-4-11
35	Formulation and Evaluation of Ascorbic Acid Effervescent Granules	P. S. Londhe, P. D. Khalate, B.P. Chaudhari , V.K. Redasani	World Journal of Pharmaceutical Research	https://wjpr.s3.ap-south-1.amazonaws.com/article_issue/1a14e4be8fa47076cb4df48d06322b13.pdf
36	Suitability Of Kinetic Energy From Footsteps For Vadjaidevi Temple At Patkhal, Taluka, District Satara	Prof. Shah. Ajinkya S, Mr. Arjun Avinash S, Mr. Khade Sagar S, Mr. Hakim Mohammadsabir N, Ms. Desai Sayali S, Ms. Mane Neha S	International Research Journal of Modernization in Engineering Technology and Science	https://www.irjmets.com/uploadedfiles/paper/issue_5_may_2022/25161/final/fin_irjmets1654414779.pdf

37	Stability Study of Different Marketed Brands of Diclofenac Sodium and Paracetamol Tablets by Using Spectrophotometric Method	A. S. Ladage, Umesh Rangat, B.P. Chaudhari , V.K. Redasani	European Journal of Pharmaceutical and Medical Research	https://www.ejpmr.com/home/abstract_id/10011
38	Post Market In-Vitro Quality Control Evaluation For Different Brands of Paracetamol Tablets Available in Indian Market	Kaustubh Jagtap, B.P. Chaudhari , A. Jadhav , V.K. Redasani	World Journal of Pharmacy and Pharmaceutical Sciences	https://www.wjpps.com/Wjpps_controller/abstract_id/17178
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41	Potentiation of Effects of Propranolol and Heparin by Antioxidant in Adrenaline Induced Myocardial Infarction in Rats	M. P. Patil, V. J. Chaware, V.K. Redasani	World Journal of Pharmaceutical Research	https://wjpr.s3.ap-south-1.amazonaws.com/article_issue/191a794e5e9d8dcdd788b938dfa82ae1.pdf
42	Evaluation of Nephro-protective Effect of DPP4 Inhibitor and Antioxidant against Gentamycin induced Nephrotoxicity in Albino Rats.	S. J. Kadam, V. J. Chaware, V.K. Redasani	World Journal of Pharmaceutical Research	https://wjpr.s3.ap-south-1.amazonaws.com/article_issue/89ee87eef6eaef52da6b771cc562a34a.pdf
43	Pharmacological evaluation of antidepressant like effect of vitamin E and its combination with amitriptyline: an acute study.	R. R. Jadhav, V. J. Chaware V.K. Redasani	World Journal of Pharmaceutical Research	https://wjpr.s3.ap-south-1.amazonaws.com/article_issue/6d77578229baf16d77017bd415e3bf7b.pdf

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45	Hepatoprotective Effect of Lycopene Against Paracetamol-Induced Hepatic Damage in Albino Rats	S. P. Pawar, V. J. Chaware, V.K. Redasani	World Journal of Pharmaceutical Research	https://wjpr.net/abstract_show/20306
46	Curcumin Potentiates Therapeutic Efficacy of Voglibose	D. B. Khandale, V. J. Chaware, V.K. Redasani , A. T. Thorat	World Journal of Pharmaceutical Research	https://wjpr.s3.ap-south-1.amazonaws.com/article_issue/72f33df90c8ac96be5a03311cbc82e0e.pdf
47	Design, Development and Evaluation of Traditional Polyherbal Formulation To Cure Dengue and Chikungunya	S. Mahamuni, B. P. Chaudhari, V.K. Redasani	European Journal of Biomedical and Pharmaceutical sciences	https://www.ejbps.com/ejbps/abstract_id/9209
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49	A Review on in situ Gel of Gastro Retentive Drug Delivery System	B. V. Aiwale, B.P. Chaudhari, A. B. Velhal, V.K. Redasani	Asian Journal of Research in Pharmaceutical Sciences	https://ajpsonline.com/AbstractView.aspx?PID=2022-12-4-10
50	The Monkeypox Virus, methods to prevent the re-emergence of the Virus	Vinay Gaikwad, R. Kothalikar, Prakash Jadhav, Pankaj Khuspe, Swapnil Phade	Journal of Advances in Bio-pharmaceutics and Pharmacovigilance	https://matjournals.co.in/index.php/JABP/article/view/1189
51	Pulsatile Delivery of Drug for a Range of Diseases	Sanket Nikam, Prakash Jadhav, B. P. Chaudhari, Atish Velhal	Asian Journal of Research in Pharmaceutical Sciences	https://ajpsonline.com/AbstractView.aspx?PID=2022-12-4-12
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54	pH Dependent Mucoadhesive In-Situ Gel Formulation Based on Abelmoschus esculentus as Sustained Release Carrier for Gastro-retentivity of Famotidine	B. V. Aiwale, B. P. Chaudhari, S. H. Deshmukh, V.K. Redasani	International Journal of Pharmaceutical Sciences Review and Research (UGC approved Scopus)	https://globalresearchonline.net/ijpsrr/v77-2/10.pdf
55	Regulatory Intelligence	A B. Velhal, Neha Nangare,	International Journal of Science and Research	https://www.ijsr.net/archive/v11i6/MR22617205617.pdf
56	Drug Development process	A B. Velhal, R. Bhosale, V.K.Redasani.	International Journal of Creative Research Thoughts	https://ijcrt.org/papers/IJCRT22A6651.pdf
57	A Review of the Preparation of Regulatory Dossiers in CTD Format and ECTD Submissions	K. A. Virkar, A. B. Velhal, V.K. Redasani	International Journal of Pharmaceutical Research and Applications	https://ijprajournal.com/issue_dcp/A%20Review%20of%20the%20Preparation%20of%20Regulatory%20Dossiers%20in%20CTD%20Format%20and%20ECTD%20Submissions.pdf
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Dettol Brand Efforts during Covid-19

Dr. Rajashri Ramesh Chavan
Associate Professor
Yashoda Shikshan Prasarak Mandal's Yashoda Technical Campus
Wadhe Phata, Satara.
rrc_mba@yes.edu.in/chavanrajashri22@gmail.com
Akash Chorge, MBA student, Yashoda Technical Campus, wadhe, Satara
chorageakash1999.ytc@gmail.com

Abstract:

The present article aimed to highlight the efforts made by Reckitt Benckiser Private Limited Company during the lockdown period. The rising demand in sanitization in Covid-19 poses an opportunity for Reckitt Benckiser to sell more Dettol. The RB has adopted various marketing strategies to sustain the label during the pandemic situation Covid-19. New labels entered the market and created a competition to establish the trusted brand Dettol. Dabur, HUL like big players, Non-branded local brands also entered into the market. Two objectives were set for the study as to understand and evaluate the efforts of Dettol during the Covid-19 pandemic situation and to identify successful outcomes during the pandemic situation. Analysis based on secondary sources e-newspaper, television commercials, published research articles, websites. The outcome reveals that during the pandemic situation, the percentage of television advertisement insertion of Dettol liquid soap was more as compared to Dettol toilet soap. 25% television ad with emotional appeal inserted for Dettol toilet soap. The Product was stretched to 'Disinfectant Spray' to exploit the favorable situation. Partnership with Tik Tok, Social website alertness, financial performance went well. Antiseptic liquid market share is largely covered by Dettol compared to its competitors. Reckitt Benckiser adopted an effective brand management process during Covid-19 through various campaigns viz. Back to School campaign, *Swachh Banega India and Maa Maane Dettol Ka Dhula. This way market got the lesson how to manage the brand in difficult situations in Covid-10.*

Introduction

Reckitt Benckiser India Ltd (RBIL) is a fully owned subsidiary of Reckitt Benckiser Private Limited Company., well renowned in India in household cleaning. An Organization operating in 60 countries, its sales in 180 countries and has had net revenues of more than $5.5 billion. Reckitt Benckiser India Ltd (RBIL) manufactures and markets a wide range of products in Personal care, Pest control, Shoe care, Antiseptics, Surface care, Fabric care,

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other categories. Amongst its many well-known brands are Dettol, Mortein, Harpic, Cherry Blossom, Lizol, Disprin, Robin powder, Colin, etc. Most of these brands are either number 1 or number 2 in their respective categories in India.

India's most trusted brand 'Dettol' is marketed as protection from germs. The rising demand in sanitization in Covid-19 poses an opportunity for Reckitt Benckiser to sell more Dettol. The RB has adopted various marketing strategies to sustain the label during the pandemic situation Covid-19. However, new labels entered the market and created a competition to establish the trusted brand Dettol. Dabur, HUL like big players, Non-branded local brands entered into the market. Thus, the market share was diluted and Dettol has the opportunity to exploit the situation but was a critical situation to protect its brand away from the competition. Therefore, interest is created to understand and analyze the efforts of Dettol to protect its market share during the pandemic situation.

Research Methodology

The present study aimed with the two objectives to understand and evaluate the efforts of Dettol during the Covid-19 pandemic situation and to identify successful outcomes during the pandemic situation. Research conducted during the lockdown period to December 2020, Descriptive study purely based on secondary sources e-newspaper, television commercials, published research articles, websites. It is analyzed through the information collected through news form and data presented with discussion and findings and conclusion.

Analysis and Discussion

Reckitt Benckiser (RB) promoted its brand Dettol in various ways like Increase Television Commercial Advertisement Insertion, Launch Dettol Spray, Launch New Range of Products with Mothers, Handwash Challenge with Tik Tok, create awareness to maintain personal hygiene during Pandemic Situation. RB used a social website platform but criticized its exaggerated claim of commercials that Dettol kills coronavirus.

RB has made 25943 insertions in the television ad during the pandemic of these Dettol Liquid soaps (13524) insertions are more compared to toilet soaps (12419). During the Covid-19 pandemic situation, various brands did a total of 46,250 insertions of an ad on television. Of these 28% insertions is of Olx, 25% of Amazon.in & 25% of Dettol Toilet Soap & 22% of Facebook.

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An Organization launched a new product '***Dettol Disinfectant spray'*** which is a one-stop solution for the germ-free home during the pandemic situation. Brand Dettol enhanced by product stretching strategy.

To make the brand strong RB's emotional appeal, "Dettol with Moms", mothers emotional & sensitive approach well worked in ad promotion during sensitive & insecure situations of covid-19.

To spread the government's message on handwashing company did a partnership with Tik Tok. The Campaign Handwash Challenge aimed to reach many people & their goal was 100 billion views at the point of a brand in India. The campaign's videos were viewed by nearly 125 billion times So, this effort was successful.

Another ad campaign used education appeal on personal hygiene to prevent and spread of Covid-19, germ protection. Reckitt Benckiser took the initiative in delivering the social message on personal hygiene. There are chances to forget the message if it is exposed one or two times so increased television commercial frequency.

Sometimes controversy also indirectly helps to show the presence of a brand. When Facebook user Andy Freeman posted an image of Dettol disinfectant spray, saying it can kill the nCoV 2019 The Dettol spray bottle label information text was it can kill cold viruses (human coronavirus and RSV) and not the nCoV 2019. This post has been read by more than a thousand times. But the authority clarified the issue in media by saying: "As this is an emerging outbreak, we do not yet have access to the new virus (2019-nCoV) for testing. Our products have been tested against other coronaviruses such as MERS-CoV and SARS-CoV and have been found to kill the virus. Although 2019-nCoV is a new strain, the virus is very similar to other coronaviruses." The firm added it will continue to work to understand the virus and test Dettol's effectiveness against it. "We are working with our partners to ensure we have the latest understanding of the virus, route of transmission and will test our product range as soon as possible," it stated.

During the lockdown, the organization took all possible measures to step up production activities. RB's offices in Makati, in the Metropolitan Manila region, were converted overnight into accommodation for more than 200 factory staff, complete with showers and canteen. The factory was still fighting to meet a Dettol demand. Every day, every week they were increasing capacity, increasing fulfillment rates. They were still under pressure to deliver. Globally the company eventually housed 1,000 workers. They put in accommodation, they arranged transport, and they arranged everything. In this position, they were making decisions which are for life or death," There were customer complaints

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on the shortage & scarcity of hand sanitizer & soaps. The Demand for Dettol was rising during the pandemic situation but Reckitt Benckiser was unable to meet rising demand due to some unavoidable & unpredictable situations e.g. CEO (important decision-maker) was locked in London flat & important production center was located in Woohan (China) center of Hot-Spot of Corona Virus in the world. This was a very adverse & problematic situation due to adverse conditions.

The performance of the Dettol brand in the Hygiene sector is more. i.e. 38% & 8% in Portfolio, 21% in both Home & Health sector, 4% Food sector and 8%.Pharma sector. There was strong consumer demand, particularly in March & April. The sale of Hygiene products increased by 12.8% and Dettol Handwash was increased by 13.6%.

The demand for Dettol increased by 62% around the world due to Covid-19. In the year 2020, the Net revenue is 6,911 £m & the gross profit is 4,212 £m. In the year 2019, net revenue was 6,240 £m & gross profit is 3,757 £m. The net income is 1,087 £m in the year 2020 which is 124 £m in the year 2019.

In 2019 during the Covid-19 pandemic situations, Dettol launched a new product Disinfectant Spray, and maintain its Dettol label through product development.

The Market share of Dettol was 83%, 10% Savlon, and 7% others. It reveals even though market share and sales increased during the covid situation but the company was unable to restrict potential competition.

According to FMCG review of Nielsen, In January & February 2020, the three most selling brands in the hand sanitizer segment (i.e. Dettol, Savlon, Lifebuoy) alone had a market share of 85% while others including existing players & smaller brands had only 15% share of the market collectively. The sale for the top three brands decreased to 39% in March 2020 as there was a sudden increase in demand for hand sanitizers but limited due to lockdown. Hence, smaller players' entry was easier.

RB introduced various campaigns as *Back to school campaign, Dettol-Banega Swachh India, Maa Maane Dettol Ka Dhula*" which was launched in the year 2014 & featured Amitabh Bachchan. RB was partnered with NDTV & Facebook to launch "*Dettol-Banega Swachh India*"- a 5-year ambitious program that addresses the rising need for hygiene & sanitization. To create awareness and importance of hygiene & sanitation, "Maa *Maane Dettol Ka Dhula*". The brand has promoted not just Dettol Original but also its variants. It makes seasonal campaigns too under this tagline which is also remembered for the longest time.

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Dettol starts to awaken customers not to consume its cleaning products, after the comments of President Donald Trump. Who suggested the possibility of injecting disinfectants to protect people from coronavirus. Reckitt Benckiser (RBGLY), a British company, warned that human consumption of disinfectant products is dangerous. It issued the statement "recent speculation and social media activity." "As a global leader in health and hygiene products, we must be clear that under no circumstance should our disinfectant products be administered into the human body (through injection, ingestion or any other route)," It shows that cognizance of media and social websites responses make the people recall.

Findings

1. During the pandemic situation, the percentage of television advertisement insertion of Dettol liquid soap is more as compared to Dettol toilet soap.

2. Total 46,250 insertions in television ads out of which 25% ads inserted by Dettol toilet soap.

3. Dettol brand enhanced by product stretching strategy through ‘Disinfectant Spray’ to exploit the favorable situation.

4. Emotional appeal in television ads became effective during the pandemic situation

5 A Platform of Tik Tok helps to increase large coverage during the lockdown.

7. Reckitt Benckiser took the advantage of social media raised controversy but immediately the responsible authority has given the statement & clarified the issue and controlled the situation.

8. Demand for Dettol was rising during the pandemic situation but Reckitt Benckiser was unable to meet rising demand due to some unavoidable situation.

9. The performance of Dettol in the Hygiene product category is more i.e. 38% compared to other categories like health, home & very least in food

10. The performance of the Dettol Brand was better than its forecast during the pandemic situation.

12. Dettol remained a trusted brand during the pandemic situation.

14. Financial performance of the brand is increased in the year 2020 as compared to the year 2019.

16. Antiseptic liquid market covered by Dettol brand compared to its competitors

17. Reckitt Benckiser was unable to meet the rising demand for Dettol sanitizer hence, Local brands jumped in the market race to fulfill the gap between increased demand & less amount supply.

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18. Reckitt Benckiser adopted an effective brand management process during Covid-19 through various campaigns viz. Back to School campaign, ***Swachh Banega India and Maa Maane Dettol Ka Dhula***

Conclusion

Brand Dettol is a trusted brand in antiseptic antibacterial agents for the safety of an entire family. Brand Dettol has come up with many products other than antiseptic liquid-like hand sanitizers, soaps, surface cleaners, Disinfectant Spray, etc. for the fulfillment of various needs of customers. In the pandemic situation of Covid-19 make compulsion for wide usage of a cleansing agent as disinfectant & sanitizers. Dettol brand got an opportunity to exploit the situation. For getting benefit from this opportunity Reckitt Benckiser took many efforts regarding Dettol brand management & increases the performance of a brand. They updated their tv commercial insertions, introduced 'Disinfectant Spray'. Thus, Reckitt Benckiser has exploited the opportunity posed during the pandemic situation due to its trusted label & continuous promotional efforts. Two challenges they faced during lockdown one is meeting rising demand and effective distribution as their production and distribution center is located in lockdown area and its marketing CEO also locked in London hotel due to pandemic lockdown so managed with digital communication.

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A Study of Online Buying Behavior of Consumers toward Standardized Products

Sarika Anil Bhosale

Asst. Prof., Yashoda Technical Institute Wadephata Satara
Email- sarikabhosale76@gmail.com Mob- 7385232600

Abstract:
In today globalized era e-commerce becomes conmen to all. Techno savvy people adopt e-commerce as it provides various facilities as it save time, save efforts such as waiting in a queue etc. But exactly what kind of products prefer by online buyers is become a matter of issues. As young generation attracts towards that e-commerce, uniquequality of may be one issue or standardized product having low consumers involvement may be prefer by consumers. Researcher selects this paper to find out factors that affect the choice of online products by online buyers. The paper may help to segment market on the basis of types of products to be preferred. Instrument is executed on 796 samples in Satara district, to find out influencing factors. Result of the research indicates that the product generally not available in local and nearby market, consumers like to shop online The Standardized products mostly having low consumers involvement are shopped on-line.

***Key Words**: Online Buying, Standardized Product, Unique Product, E-Commerce*

Introduction:

Globalization brings quick and rapid access of all things around the world. In today's modern era person do not have much time to visit at various showrooms and made shopping. This problem was addressed by E-shopping. It is the concept of electronic shopping means to shop online using internet from anytime, anywhere.

E-shopping means act of purchasing product or services over the internet. Online shopping has grown popularly over the years, mainly because people find it convenient and easy to bargain from the comfort of their place, home or office. An important benefit e-shopping provide is that unlike traditional shopping there is no need to wait in long lines or search from store to store. It is just search ofan advertise by Google.

E-commerce, which stands for electronic commerce, refers to the exchange of products and services over an electronic network, such as the phone or the Internet. It refers to a website that accepts credit card payments and sells goods or services directly from the site utilising a shopping cart or shopping basket system online. It entails carrying out business using electronic

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media and information technology, including electronic data interchange (EDI). In simple words, electronic commerce involves buying and selling of goods and services over the World Wide Web. Customers can purchase anything at anytime right from a car or a cake sitting comfortably at their location and gift it to someone sitting miles apart just by click of a mouse.

Literature Review:-

A plethora of search scholar studied on the online shopping among national and international level. Adoption of online shopping still observed on nascent stage. The researchers seem to take different perspectives and focus on different factors in different ways.

Researcher has attempted contextual review of articles published in international, national and regional research journals.

Most of these studies have attempted to identify factors influencing or contributing to online shopping attitude and behavior, few of them also focus their attention on demographic difference in online shopping behavior. The researcher seems to take different perspective by different ways viz. (Haq, 2010) Author opine that the perception of online shoppers is independent of their age and gender but dependent of their qualification & gender and income & gender. Further more (Ahasanul Haque, 2006), reported that gender and family income had significant relationship with overall attitude. (Almousa, 2011)revealed that in 18-25 years age groups, both males and females, use the internet heavily and more adapted to internet shopping. Although this age group does not have higher incomes of their own and is not expected to earn income yet in the Saudi culture, rather, they are mostly dependent on others until they graduate and then participate in the job market.

On contrary (Srikanth Beldona, 2011) didn't observed any significant difference between male and female online buyers. This result is also supported by (Nabil Tamimi, 2004) that gender and frequency of online shopping are independent of each other. Also (Yet Mee Lim, 2010) did not find any statistical significant gender differences in online behaviours and Attitudes.

Again on contrary (Jooyoung Park, 2009), opine that as compare to male females were search more information by visiting more product pages in the online shopping process. Author agrees that female are more interested in clothing and males are more interested in electronics goods category. He also opines that females are more likely to read the reviews on products or services and seeks the help on an assistant's agent for online shopping. Specifically, females consulted

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customer reviews and used an assistant agent more often when shopping for experience goods than when shopping for search goods. On the other hands, males showed no significant differences in information search across product categories. This implies that the influence of product characteristics on consumers' information search differs between males and females. A consistent result is also observed by (Arpita Khare, 2011) The male and female students differed in their attitude toward online shopping, utilitarian motives, and purchase intention. Men are likely to perceive online shopping Web sites as convenient, flexible, enabling product, price comparisons, and easy to operate.

(Acilar, 2012), reported that male students have more positive attitudes toward online shopping than female students , consistent result revealed by (Ms. Asmatara Khan, 2012) Among the entire population of internet users, men more than women are inclined to trying the internet for varied reasons.

1.2 Research Problem :

India's economic growth has accelerated significantly from last two decades and it has inflated the spending power of its citizens. With rising incomes, household consumption has increased and a new Indian middle class has emerged. The world is changing very fast. Technosavy people don't have time to west on shopping. Their trends towards adopting new technologies of shopping were increasing.

Estimate of internet users 'universe' includes those accessing internet on their mobile phone. Users are also profiled as consumers of a variety of product and services. E-shopping now a days provides variety of produce viz. FMCG product, Wearable, Household and kitchen durables, Automobiles, Electronics, Mobiles, and various services.

Changing Attitude towards Online Shopping

Despite the proliferation of "Awareness, Future Demand Emphasis for Developing Markets & Present Problems" malls, individuals still prefer to shop online. Modem consumers are more sensible and able to access the market's options. With the internet, consumers are made aware. Every day, more people are using the internet, which draws in customers who can shop online. It was never anticipated that Indians would use e-commerce in this manner. Ticketing, trip bookings and even books and movies appear fine to buy online. Knowing that in India sizes vary

from brand to brand and quality is inconsistent, even for some of electronic items, how is it that there are people buy these items online? In India there are few segments of people who have not yet tried purchasing over internet.

Hypotheses of the Study:

Standardized product can be defined as the product which produces with the process of setting generally uniform characteristics for a particular good or service. Product standardization among the goods provided by different businesses operating in technology-based industries can be useful for consumers since it permits competition among the various suppliers.[1]

A standardized product means a good quality product and branded product, as online buying lacks in physical teach and feel approach. Generally standardized products are more prefer by the consumers hence following parameters are sought to judge the consumers behavior.

1. The Standardized products mostly having low consumers involvement are shopped on-line.

Objectives of the Study:

1. To find out factors influencing purchase decision regarding e-shopping.

Research Methodology:-

The study is conducted in Satara District State of Maharashtra, India. Study limited to the buyers from various locations in Satara district. Samples are selected from 11 taluka places in Satara district vary in numbers. Structured schedules are the instrument for data collection.

Data has processed using MS-Excel and analyzed using SPSS Package. Descriptive analysis, inferential statistics and multivariate statistical tools brought into use.

Data Analysis:

Nature of product prefers to buy online

Standardized Product

Following table shows the agreement of samples towards buying online nature of as a standardized product. Six parameters were asked to option on five point likert type scale. 1 for

1. [1] Read more: http://www.businessdictionary.com/definition/product-standardization.html -#ixzz36h7wLiwY

strongly disagree and 5 for strongly agree. The options were analyzed using mean, SD and ranks calculated on mean score as follows.

Table 1
Standardized Product

(n=796)

Sr.	Standardized Product	Mean	SD	Rank
1	I like to buy popular brands via online shopping.	4.58	0.686	1
2	A popular brand means good quality products.	4.38	0.632	2
3	Internet shopping provides a better qualityproduct.	4.03	0.925	8
4	I would like to pay more for branded product.	3.95	0.995	6
5	It is important for me to buy products/services with popular brand names	4.2	0.797	3
6	If I buy products/services from a web-retailer, I would prefer to buy popular brand name.	4.15	0.777	4

(Source: Field Data)

Table 1 presented above reveals that the samples prefer to purchase standardized product online. Customer involvement is low in case of standardized and branded products; hence samples had given highest preference i.e. 1st rank to buy popular brands with 4.58 mean. A quality is important parameter which makes brand popular is next preferred by samples with 4.38 mean and 2nd ranks. Samples had given 3rd rank to "It is important for me to buy products/services with popular brand names" with 4.2 mean. Remaining parameter having their mean values more than 3.96 it means that samples are agreed to buy standardized or branded products online.

Unique Product

Following table shows the agreement of samples towards buying online nature of as a unique product. Four parameters were asked to option on five point likert type scale. 1 for strongly disagree and 5 for strongly agree. The options were analyzed using mean, SD and ranks calculated on mean score as follows.

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Table 2
Unique Product

(n=796)

Sr.	Unique Product	Mean	SD	Rank
1	I prefer to buy unique product via online shopping	4.3	0.844	1
2	The product generally not available in local and nearby market, I prefer to shop online.	4.24	0.73	2
3	New arrivals / products are quickly available online	4.16	0.816	3
4	I like to introduce new style	3.82	0.951	4

(Source: Field Data)

Table shows that unique product offered by online retailer attracts most of the samples as that parameter secured 1st rank with 4.30 mean. "The products generally not available in local and nearby market, respondent prefer to shop online," this parameter secured 2nd rank to with 4.24 mean. Respondent believes that new arrivals/ products are quickly available online as it scored 3rd rank with 4.16 mean and parameter 'I am one who tends to introduce new style' secure low rank with 3.82 mean.

Inferential Analysis:

H0: All types of products are shopped online.

H1: The Standardized products mostly having low consumers involvement are shopped on-line.

Samples were asked to rate their opinion on statements representing standardized products and unique products. Six statements were representing standardized products and four statements were representing unique products. The opinions were sought on five point scale. The mean score has calculated and the series of mean score of opinions of standardized products and unique products have put to test for test of significance.

Description of type of product shopped online

The nature of product shopped online has been assessed as follows. Two type of products were ask to opine on one is standardized product and another is unique product.

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Table: 3
Description of type of product shopped online
(n=796)

Sr.	Particulars	Mean	N	S.D.	SE Mean
1	Standardized Product	4.210	796	.522	.0185
2	Unique Product	4.13	796	.599	.021

Source: (Field data processed)

Above table shows that the opinion of samples means score for standardized product is 4.2 with standard deviation 0.522 and that of mean score for unique product is 4.13 with standard deviation of 0.599. It has observed that the figures of mean score and standard deviation are almost same.

Type of product shopped online test of significance

Following table shows test of significance regarding opinion of samples towards shopping standardized products and unique products. Paired sample't' test has used to test the significance.

Table: 4
Type of product shopped online test of significance
(n=796)

		Paired Differences					t	df	Sig. (2-tailed)
Sr	Particulars	Mean	SD	SE Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
1	Standardized Product and Unique Product	0.0887	.6605	.0234	.04281	.1347	3.792	795	.000

Source: (Field data processed)

Above table shows the value of calculated paired't' is 3.792 with a 'p' value 0.000 the test is significant hence **null hypothesis is rejected and alternative hypothesis is accepted**. The alternative hypothesis is The Standardized products mostly having low consumers involvement

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are shopped on-line. In this test the merely opinions of samples towards standardized products has compared with opinions of same samples towards unique products.

Looking at the mean difference value is 0.088 with a standard deviation 0.66 shows proximity of the opinions of samples towards statements related with standardized product and unique product.

Findings:-

1. Customer involvement is low in case of standardized and branded products; hence samples had given highest preference i.e. 1st rank to buy popular brands with 4.58 mean. A quality is important parameter which makes brand popular is next preferred by samples with 4.38 mean and 2nd ranks. Samples had given 3rd rank to "It is important for me to buy products/services with well-known brand names" with 4.2 mean. (Refer Table No. 1)
2. Unique product offered by online retailer attracts most of the samples as that parameter secured 1st rank with 4.30 mean. "The product generally not available in local and nearby market, Samples like to shop online," this parameter secured 2nd rank to with 4.24 mean. Samples believes that new arrivals / products are quickly available online as it scored 3rd rank with 4.16
3. It is opined that the opinion of samples means score for standardized product is 4.2 with standard deviation 0.522 and that of mean score for unique product is 4.13 with standard deviation of 0.599. It has observed that the figures of mean score and standard deviation are almost same.
4. It is observed that the value of calculated paired 't' is 3.792 with a 'p' value 0.000 the test is significant hence **null hypothesis is rejected and alternative hypothesis is accepted**. The alternative hypothesis is The Standardized products mostly having low consumers involvement are shopped on-line.

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RESEARCH ARTICLE

BUYER'S PERCEPTION OF E-VEHICLE IN SATARA

***Dr. Rajashri Ramesh Chavan and Mst. Shubham Siddeshwar Jadhav**

Yashoda Technical Campus, Satara, Maharashtra, India

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****Corresponding Author:***
Dr. Rajashri Ramesh Chavan

ABSTRACT

The paper aims to understand the awareness of customers and government efforts to promote e-vehicle and also to understand customers' product perception. This study is conducted in Satara city with 125 samples which consist of both existing customers and potential customers. A stratified disproportionate sampling technique is adopted to select the sample. The Schedule is designed to collect the feedback from the sample. The nature of the research study is descriptive. The study identified and evaluated the consumer perception of various factors about the electric bike. The result reveals that Government is taking rigorous efforts through FAME Amendment and PLI Scheme for the Auto sector. Satara customers are well aware of e-vehicle. There is a growth in both e-bikes and e-cars in Maharashtra. There is a combination of both positive and negative perceptions about e-vehicle. Most of the respondents consider the cost and the mileage in purchasing a bike, so there is ample potential for an electric bike in two-wheeler sectors. But their battery performance, speed, and appearance are the major factors that are affecting the sales of electric bikes.

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INTRODUCTION

In a thrust toward incentivizing new-age technologies and fulfilling the policy taken at COP26 to reduce its carbon emissions to zero by the year 2070, India is aggressively promoting the adoption of Electric Vehicles (EVs). India aims to switch 30 percent of private cars, 70 percent of commercial vehicles, and 80 percent of two and three-wheelers to Electronic Vehicles by the year 2030. For this, both Central and state governments are offering various incentives to buyers and manufacturers. Electric scooter sales touched double digits in the recent period. They could already reach one million sales, had it not been for the Russia-Ukraine war, which has led to a shortage of semiconductors and other materials. According to the source of published news, Last year, 0.2 million electric scooters were sold. Analysts estimate that the two key players Ather Energy and Ola Electric will jointly sell between 0.25 million to 0.26 million scooters this year. By 2023 the expectation based on the capacity built up by manufacturers is that sales could range between 1.5.2 million, making a further dent in the overall two-wheeler market. If that happens, electric scooters will cross another milestone, they will account for 10% of the total 18-20 million per annum two-wheeler market, which includes motorbikes. It could also mark a shift in the domestic market, where 70% of two-wheeler sales come from motorcycles, in favor of electric scooters in the coming years. Apart from anything else, there are only a few electric bikes and these have just entered the market. The government has its estimate. It believes that 80% of the bikes will be electric by 2030. We estimate that about 0.7-0.8 million electric scooters will be sold in 2022. Currently, there are 35-36 players but it expects consolidation in the next two to three years. The market will grow gradually. The Price of electric scooters is also likely to go up when the government eventually withdraws the subsidy, which is currently helping to keep its price low. Perception matters a lot when anyone in the business world. Needless to say, companies that enjoy favorable customer opinions are often the ones that regularly break the ceiling and achieve remarkable success year in and year out. On the other hand, businesses with poor customer perceived value find it hard to maximize their effort, struggle to realize the true potential, and in most cases, also get consigned to obscurity. So, one should always strive to meet the expectations of customers and want to be seen in a positive light, and maintain a desired level of perception. Consumer perception is vital for any business for many reasons. Perception builds trust, Perception propels sales, Perception creates reputation, and Perception drives key metrics Perception generates word of mouth. Customers often form an opinion about a product based on many factors and not all will be in your control. If you have the right customer experience strategy, you can easily work on most things that shape or break the perception and bring the desired improvement to the result. Similarly, there are so many aspects such as price, quality; positioning, etc. that can decide how your business is seen by others in the market.

Review of Literature

(Ashok, 2019)This article highlight the importance of E-Vehicles and the efforts of the government in implementing policies to promote E-Vehicles to reduce the dependence on oil, decrease greenhouse gasses and improve air quality.

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The study was conducted in Bangalore city. The study analyses the awareness levels of customers on government initiatives for E-transportation in India. (Sanguesa Julio A, 2021)This paper reviews the advances of EVs regarding battery technology trends, charging methods, as well as new research challenges and open opportunities. (Hannan M A, 2014)This paper highlights existing technologies are more or less capable to perform HEV well; however, the reliability and the intelligent systems are still not up to the mark and also highlighted many factors, challenges, and problems with sustainable next-generation hybrid vehicles.(Garling Anita, 2001) Author outlines a two-phase strategy for the marketing of Electric Vehicles (EVs) based on a discussion of current and expected future characteristics of EVs and a review of research on early adopters.

RESEARCH METHODOLOGY

The research study is conducted in Satara city from Nov 2021 to Jan 2022 with the objectives of understanding the awareness of customers and government efforts to promote e-vehicle and knowing the product perception of existing and potential customers and to know the price perception and identifying the influencing factor in buying. An unequal proportionate stratified sampling technique is used to collect feedback from a total of 125 samples.

A Schedule is used to collect the opinion of respondents. Both actual buyers and potential buyers are selected as sample units. Collected data analyzed with the help of descriptive analysis and presented with tabulation and described through data analysis and discussion. The nature of the study is descriptive. Results are presented in the form of findings and classified data and tabulation presented in the annexure.

Data Analysis and Presentation: A Researcher has analyzed the collected data and discussed it as follows. Published data talks about the growth of e-vehicles in Maharashtra in cars and two-wheelers. It shows as follows. The above figures depict that E-cars and two-wheelers are increasing in Maharashtra. Of these two-wheelers, growth is higher compared to e-Cars in Maharashtra.

Table 1. E- Vehicles (cars and 2-wheelers) Growth in Maharashtra

Sr.	Year	Cars	2-wheelers	Registered
1	2019-20	183	5479	7400
2	2020-21	1128	6875	9415
3	2021-22	2633	19396	23786

Source: https://timesofindia.indiatimes.com/city/mumbai/e-vehicles-in-maharashtra-up-153-in-1-year-more-than-double-in-mumbai/articleshow/88555385.cms

It reveals that acceptance of e-vehicles are increasing day by day in Maharashtra. The government's efforts in implanting e- vehicles policies are getting success to some extent. It is a very good time for electric vehicles in Mumbai and Maharashtra. While the state saw a phenomenal 153% rise in new e-vehicle registrations in the first nine months of the financial year 2021-2022, the city recorded a growth of 112% in the same period, the latest transport statistics show. Compared to 9,415 e-vehicles registered in 2020-21, the number of registrations skyrocketed to 23,786 in just nine months of 2021-22 (April 1 to December 27 this year).

Government Efforts: India is aggressively promoting the adoption of Electric Vehicles (EVs). For this, both Central and state governments are offering various incentives to buyers and manufacturers.

PLI Scheme for Auto Sector: In September this year, the Union Cabinet approved a Rs 26,058 crore production-linked incentive (PLI) scheme to accelerate domestic manufacturing of electric and fuel cell vehicles and drones in India. As per the government's estimate, the scheme would attract Rs 42,500 crore in fresh investment in the automobile and auto components industry over five years. The government has allocated Rs 25,938 crore for the automobile sector and the remaining Rs 120 crore for the drone sector

FAME II Amendment: FAME-II (Faster Adoption and Manufacturing of Electric Vehicles-II) scheme. Under this, the government significantly reduced the price gap between petrol-powered two-wheelers and electricians by increasing the subsidy rate for electric two-wheelers from Rs 10,000/kWh, to Rs 15,000/kWh, while also capping the incentives at 40 percent of the cost of vehicles as against 20 percent earlier. Government official plans were afoot for 1500 new EV charging stations across the Mumbai region. The government also wants to ensure that 10% of new vehicle registrations by 2025 are electric vehicles.

Perception of Customers in Satara City: The Researcher collected the opinion of customers in Satara city to examine their perception of customers. After analysis of the collected data, it shows that 60% of respondents have bought an electric bike and 40% are potential buyers. Of these respondents, 75.2% of respondents are aware of electric bikes, and very few i.e. 24% are unaware. This percentage is due to existing customers' samples being more than potential.43.2% of the sample perceive that the price of a vehicle is high, 26.4% perceive low, and 5.6% only perceive very low. There is mixed opinion about the price of Electric Bike. There is a need to plan an effective convincing price aspect for Electric Bike. 36.8% perceives mileage of vehicle is 'Very Good', 24.8% perceive 'Good', 28% perceive 'Bad'& 10.4% perceive 'Very Bad'. It reveals that the perception of customers towards mileage of the electric bike is satisfactory as they perceive (61.6%) 'Good' rest i.e.39.4% perceives 'Bad'. 40.8%sampleperceive the speed of the vehicle high, 11.2% perceive very high, 22.4% perceive low & 25.6% perceive very low. Thus, the perception of samples towards the speed of the electric bike is satisfactory.

Respondent (53.4%) preferred Electric Bike price range between 50,000-90,000 & rest i.e.46.6% preferred the price range between90000-110000. Zero Emissions and Environment & Tax Benefits are two important criteria that make respondents purchase Electric Bike.(66.66%)samples are dissatisfied with their post-purchase experience & (33.33%) customers showed satisfaction towards post-purchase experience. It reveals that 66.66% are dissatisfied with the post-purchase experience**.** There is a need to identify the reasons for their dissatisfaction. There is further scope to study customer satisfaction towards Electric Bike. (60%) sample perceives the high cost of maintenance and (40%) perceives low-cost maintenance of e-bikes. There is a need to identify the reasons for their high-cost maintenance. There is further scope to study customers' high-cost experience with Electric Bike.54.66% samples said e-bike speed is 'average '& (26.66%) samples said 'good' speed & (18.66%) said 'poor'. There is a need to improve the speed of the Electric Bike. When respondents talk about mileage coverage per charge, (52%) samples said they cover 90-110 km mileage per charge & (30.67%) cover110-130km,(12%) said 70-90km & (5.33%) very few customers said mileage covered per charge is above130km.It reveals that the majority of sample cover distance is 90-110km per charge. A very few (5.33%) customers said the distance covered per charge is above 130km. So there is a need to study more on their power-saving storage of batteries to cover long distances per charge.

RESULTS

Results of a study found after analyzing the data where 60% samples are existing users of Electric Bike & 40% are willing to buy i.e. potential customers. There is a mixed non-proportionate group of samples used for the study. The results are based on their opinion. The majority of respondents are aware of electric bikes. There is mixed opinion on the perception of price. Perception of about mileage of the electric bike is satisfactory as they perceive (61.6%) good & rest i.e.39.4% perceive bad. The perception of customers towards the speed of the electric bike is satisfactory. Respondents are more convinced to buy Electric Bike for low running features than other features. The preferred price of a vehicle ranges from 50000 to 90000. It shows respondents are not ready to spend more. Zero Emissions and Environment & Tax Benefits are two important criteria that make

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respondents purchase Electric Bike. All sources viz. The Campaign, advertising, pamphlet, & others are used by the customer to know the Electric Bike. Respondents gave more preference to advertising the product. Actual customers are more preferred the campaign & Potential customers prefer the pamphlet. The majority of respondents are dissatisfied with the post-purchase experience**.** There is a need to identify the reasons for their dissatisfaction. It is well said that dissatisfied customers are always more dangerous than satisfied customers. There is further scope to study customer satisfaction towards Electric Bike. The majority perceive the maintenance cost of the vehicle as high**.** There is a need to identify the reasons for their high-cost maintenance. The majority perceive the speed of the vehicle to be average as per their post-experience**.** It reveals that customers get attracted by vehicle speed. So to attract more customers need to improve the speed of the vehicle. The majority of samples covered distance is 90-110km per charge and very few covered above 130km. Therefore, there is a need to examine power-saving storage i.e. battery to cover long distance per charge. Respondent thinks appearance plays a major role while making purchasing decisions so the Electric Bikes need to be made more attractive.

CONCLUSION

To be conclude that the maximum number of respondents are not aware of Electric bikes. Thus, it requires various promotional activities to increase the awareness level & thereby increases the sales.

The study also identified and evaluated the consumer perception of various factors about the electric bike. The result of this study shows that there is a both positive and negative perception about e-vehicle. Here most of the respondents consider the cost and the mileage while purchasing a bike, majority of customers cover distance is 90-110km per charge so there is ample potential for the electric bike. But their battery performance, speed, and appearance are the major factors that are affecting the sales of electric bikes. The study explains the perceptions prevailing in the minds of customers and highlights the areas to improve the e-bike in near future.

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Appendices

Table No. 1 Distribution of Respondents As Per Their Type of Customer

Sr. No	Type of customer	Frequency	Percentage (%)
1	Actual customer	75	60
2	Potential customer	50	40
	Total	125	100

(Source:-field data)

Table No: 2 Awareness of Electric Bike

Sr. No	Parameters	Frequency	Percentage (%)
1	Yes	94	75.2
2	No	31	24.8
	Total	125	100

(Source:-field data)

Table No: 3 Respondent Perceptions Towards Price Of Electric Bike

Sr.	Perception	Types of customer				Total	
		Actual customer		Potential customer			
		Freq	%	Freq	%	Freq	%
1.	Very High	19	25.33	12	24	31	24.8
2.	High	29	38.67	25	50	54	43.2
3.	Low	22	29.33	11	22	33	26.4
4.	Very low	05	6.67	02	4	07	5.6
Total		75	100	50	100	125	100

(Source:-field data)

Table 4 Criteria Used to Prefer Electric Bike

Sr.	Parameters	Weighted Average	Rank
1	Insurance of registration cost	123	3
2	Petrol Consumption	123	3
3	Tax Benefits	124	2
4	Zero Emissions And Environment	125	1

(Source:-field data)

Table No 5. Perceptions towards Speed of Electric Bike

Sr.	Opinion	Types of customer				Total	
		Actual customer		Potential customer			
		Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
1.	Very High	08	10.66	06	12	14	11.2
2.	High	29	38.66	22	44	51	40.8
3.	Low	9	12.02	19	38	28	22.4
4.	Verylow	29	38.66	03	6	32	25.6
5.	Total	75	100	50	100	125	100

(Source:-field data)

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Table No. 6 Perception of Customer Towards Sources Used To Know Electric Bike

Sr.	Opinion	Types of customer				Total	
		Actual customer		Potential customer			
		Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
1.	Campaign	22	29.33	07	14	29	23.2
2.	Advertising	32	42.67	22	44	54	43.2
3.	Pamphlet	10	13.33	14	28	24	19.2
4.	Other	11	14.67	7	14	18	14.4
5.	Total	75	100	50	100	125	100

(Source:-field data)

Table 7. Perception Of Customer Towards Mileage Of Electric Bike

Sr.	Opinion	Types of customer				Total	
		Actual customer		Potential customer			
		Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
1.	Very Good	39	52	7	14	46	36.8
2.	Good	10	13.33	21	42	31	24.8
3.	Bad	16	21.34	19	38	35	28
4.	Very Bad	10	13.33	03	6	13	10.4
5.	Total	75	100	50	100	125	100

(Source:-field data)

Table No: 8 Respondent Perceptions Towards Feature To Convinced To Buy Of Electric Bike

Sr.	Opinion	Types of customer				Total	
		Actual customer		Potential customer			
		Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
1.	Low weight	11	14.67	09	18	20	16
2.	Low running	39	52	25	50	64	51.2
3.	Registration onnot required	19	25.33	14	28	33	26.4
4.	Others	06	8	02	4	08	6.4
5.	Total	75	100	50	100	125	100

(Source:-field data)

Table No: 9 Respondent Perception towards Price Range Preferred

Sr.	Price Range	Types of customer				Total	
		Actual customer		Potential customer			
		Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
1.	50000- 70000	04	5.33	09	18	13	10.2
2.	70000- 90000	29	38.67	25	50	54	43.2
3.	90000- 110000	34	45.33	14	28	48	38.4
4.	110000-&above	08	10.67	02	4	10	8.2
5.	Total	75	100	50	100	125	100

(Source:-field data)

Table No: 10 Respondent Perceptions Towards Over All Post Purchase Experience

Sr no.	Opinion	Frequency	Percentage (%)
1.	Strongly dissatisfied	21	28
2.	Dissatisfied	29	38.66
3.	Neutral	18	24
4.	Satisfied	07	9.33
5.	Strongly satisfied	0	0
Total		75	100

(Source:-field data)

Table No. 11 Perception Towards Over All Post Mileage Experience

Sr no.	Opinion	Frequency	Percentage (%)
1.	Good	20	26.66
2.	Average	41	54.66
3.	Poor	14	18.66
Total		75	100

(Source:-field data)

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Table No. 12 Perception Towards Over All Mileage Covered Per Charge

Sr.	Opinion	Frequency	Percentage (%)
1.	70-90 Km	09	12
2.	90-110 Km	39	52
3.	110-130 Km	23	30.67
4.	130-Above	04	5.33
Total		75	100

(Source:-field data)

Table No. 13. Perception of Maintenance Cost of Electric Bike

Sr.	Opinion	Frequency	Percentage (%)
1.	Low Cost	30	40
2.	High Cost	45	60
Total		75	100

(Source:-field data)

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Study of Factors affecting the National Anonymously: Dark Web

Akshay Sankpal[2], Chetan Shivankar[1], Priyanka Pawar[3], Pranjali Gade[4]
[1, 2, 3, 4]*Yspm Satara - MCA Department, Shivaji University Kolhapur*

Abstract: This paper talks about how the dark web is utilized to genuine purposes just as to hide the noxious exercises or criminalism. The Dark Web is at the focal point of the discussion about whether online namelessness ought to be kept up despite the criminal behavior that it empowers. This paper will limit its degree by concentrating exclusively on the national contemplations of the Dark Web, and not those issues that dig into the domain of local law implementation. Drug dealing, firearms, fake products, unlawful erotic entertainment, and so forth these are issues that this paper characterizes as falling into the domain.
Keywords: Dark web, security, social media, cyber attacks

I. INTRODUCTION

The dark web frames a little piece of the profound web, the piece of the Web not recorded by web indexes, albeit here and there the term profound web is generally used to imply explicitly to the dark web. The dark Web is portion of reflective Web which has been intentionally enclosed, is blocked over ordinary Web programs. While having the network has associated the whole world readily available and has upset how tasks occur all through the world, some may contend that the web has brought more damage than anything else

Cyber attack targets are usually aimless: unselective, however in modern times, targeted attacks that too without any purpose are made. It is believed that such attacks are made for a specific purpose. In these present times, it is thus problematic to avoid or even stop all the cyber attacks, even when we have taken security measures. They are often same that they have a tendency to area unit in a very defense solely state of affairs. So as to beat this example, it is essential to foresee the cyber attacks and to involve applicable security methods ahead. It is essential to apply risk intelligence that permits this. In common, several aggressors share data and tools accessible for attacks in special societies on the dark web. This is the reason, it is supposed that there is huge volume of threat intelligence on web.

Thus by means of consuming the intelligence, we are able to observe cyberattacks ahead and develop a active defense.

Dark web excavation is a quickly developing area for research. Web mining systems are also utilized to distinguish as well as avoid dread pressures brought about by pirate terrorists everywhere throughout the world. Nowadays, these attacks ahead and develop a active defense .

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The expression "social media" states that web boards that permit the trading of User-Generated Content. They incorporates different accessible correspondence stages, for example, gatherings and websites, content networks, for example, Youtube.com and Flickr.com, and internet business networks, for example, eBay.com and Amazon.com. The fundamental source of a lot of threat knowledge is the dark web gathering, the profound dark web discussion can more readily understand the intentions and exercises of illegal components. A deep understanding of these networks will incredibly help keep up cybersecurity and empower cybersecurity professionals to more readily comprehend their adversaries.

Dark web excavation is a quickly developing area for research. Web mining systems are also utilized to distinguish as well as avoid dread pressures brought about by pirate terrorists everywhere throughout the world. Nowadays, these terrorist tackles exist greatest issue intended for the humankind. These terrorists are individuals who plan, take an interest in, and accomplish some actions of terrorism

The anonymity of the user details such as IP address and location of the user is disclosed to the world. This paper throws a light on how dark web is used for destructive purposes and how it has destroyed the integrity of nation through its activities and usage.

II. LITERATURE REVIEW

Embedded inside the Deep Web is the Dark Web or Dark Net. It's here that terrible on-screen characters where everything being equal content kiddies out to ruin sites, professional programmers who break into corporate and government systems to take information, unleash devastation, and commit extortion; drugs, arms, and human traffickers, arranging attackers and planning and digital pirates--speak with each other and exchange hacking tool, malware, ransom- ware, and different illegal tools and services. This secret market is huge enough to contain its very own web crawlers, network gatherings, and rating frameworks and systems simply like the WWW. Such pirates and terrorists namelessly set up different sites inserted in the open Internet, trading belief system, spreading purposeful publicity, and selecting new individuals.

Social media allows exchange of different content under the User-Generated Content (UGC) which comprises numerous available communication platforms such as media and blogs, contented group of people and e-commerce websites.

Because of the secrecy of the dark system, numerous illegal components have carried out unlawful violations on dark web. It is tough to create law requirement authorities to follow the character of these digital culprits utilizing conventional system overview methods dependent on IP addresses. The risk data is primarily from the dark web discussion and the dark web advertise. Late years have seen a flood in investigations of clients' effect in online networking, as promoting writing has demonstrated that clients' influence impacts basic leadership.

Dark Web destinations is just like a stage for Internet clients to whom insignificance is basic, since they give assurance after unauthorized users, thus in addition for the most part incorporate encryption to counteract observing. A moderately well-known way for constituent that lives on the dark Web originate in the Tor organize. The Tor system is an unknown system that must be acquired with a unique Web code, called the Tor program (Tor 2014a).

Initially appeared as The Onion Routing (Tor) venture in 2002 by the US Naval Research Laboratory, it was a strategy for imparting on web namelessly. An additional system, 12P, gives a considerable lot of similar highlights that Tor does. Be that as it may, 12P which then intended to be a system inside the Internet, with crowd remaining restricted in its outskirts, Tor provides better mysterious access to the open Internet and 12P gives an increasingly powerful, solid "arrange inside the system" (Tchabe and Xu 2014).

III. RESEARCH METHODOLOGY

AADHAR DATA LEAK: EDWARD SNOWDEN BACKS INDIA REPORTER OVER EXPOSE.

An Indian Journalist was accused for committing criminal offence and buying the Aadhar details. US whistle-blower Edward Snowden had tweeted in provision of an

Indian reporter being inspected by police department for a explosion on the debatable Aadhaar biometric identity pattern. He a foresaid that Rachna Khaira, who aforesaid she was capable to acquire residents' private information for simply five hundred rupees ($8: £6), be worthy of a present Identification authorities say she committed a "criminal offence" by accessing the Aadhaar information . The editor of tribune newspaper, that employs Ms Khaira , defended the report, expressed it had been revealed "in response to a extremely real concern among the citizens on a matter of great public interest"

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1) Ransom-ware As a Service (RaaS) Ransom-ware-as-a-service (RaaS) is slightly completely different. Not like traditional ransom-ware, RaaS doesn't need the attacker to be essentially trained at writing code to launch attacks. That is as a result of the RaaS delivery model is comparable to a monthly subscription service. This type of affiliate program creates a win- win situation for every the malware author and additionally the subscription customer. There is generally some form of percentage or split between the two parties that's typically set up front. In the end, the only loser is that the victim who pays the demanded financial ransom in hopes of safely getting their valuable knowledge back. RaaS is that it removes an oversized barrier to dangerous actor's entry into this field. The ability to code was once a demand for hackers that wanted to act unwell can upon society. Although, there is no honor amongst thieves, there was a loose understanding that if a victim paid the ransom in most cases their information would be returned or decrypted back to normal. There were numerous high-profile breaches regarding famous websites and on-line offerings in current time, and it was quite probable that a number of money you owed must have been impacted. It's also possible that your identification are indexed in big record that's moving across the dark web Secuirty researchers at 4iQ spent their days observing the wide spread dark net places, hacker media, and online black markets for disclosed records. The most current located: a 41- gigabyte document which consisted of an incredibly around a billion of authentication mixtures i.e Username and passwords. The absolute size of the data is terrifying enough, however there is a lot of such data present yet.
2) More than 45,000 people created the online network and traded offensive pictures of kids and various videos on a dark- web medium that was solely reachable over a particularly encrypted browser. Additional approach, known as Operation Onymous, disclosed a bove four hundred "hidden services" in a trial by totally seventeen different countries which was co-ordinated by police force, also the FBI. This procedure headed to a lot of money of Bitcoin being taken over where seventeen arrested.
3) The Chloe Ayling case Chloe Ayling, British model who is 20-year-old was seized by a infamous sex the 'Black Death Group' which is a sex trafficking gang and was trapped in Milan. The captive was controlled by the mother of one the person for 6 days in a distant house being which led to fake guarantees of a photo-shoot. During the auction occurred she was narcotized and stuffed in a bag on the dark web. A shadowy actively accessible groups also known as 'the black group death' have connected many kid trafficking instances. It is claimed that the people accessing the dark web shops for the girls who have being kidnapped across Europe.

III. CONCLUSIONS

In this paper, we tried to put on the darker side of the the dark web. In the most well-known Dark Web markets, unlawful things are frequently sold, for example, illegal drugs, malware, firearms, stolen credit cards, and banking data. Digital assault administrations and cyber- attacks are much of the time sold in these equivalent markets, similar to access to botnets that can direct have access to Distributed denial of Services (DDoS) so as to briefly harm the websites. Websites like the ones your business may have on the clear net. You can shield your organization from that harm by sending the typical sorts of safety efforts and devices Use antivirus programming, log your system gadgets, send SIEM, arrange firewalls, switches, know about the majority of your system movement, utilize powerful identity and client get to the other access managements, encrypt your information and stored data and so on.

Even the youngsters are growing their interests in the dark web access. The anonymous behavior of the dark web has it possible to hack the details about the people from social media as well. Websites which are hosted under the TOR browser provides no identity to its user. And hence no traces are left behind. Even if someone tries to track the activities of such pirates the encryption is of such a high level that just a few layers of the Onion Routing can be decrypted and it requires a lot of experimentation. Social media such as Facebook and twitter are the ones which have tremendous threat of getting hacked and the personal details of the users can easily sold. To overcome this, safety measures must be taken. One should shield their personal data and keep them encrypted.

Corporate data breaches are becoming alarmingly more frequent, and cyber criminals will often try to sell that data on the Dark Web. That data could consist of login credentials or financial information which can be used to do tremendous harm to your business when in the wrong hands.

The Dark web presents a significant security risk. As a result of its unique characteristics, like name lessness, cybernetic markets, and which in turn uses the cryptocurrencies, in this network, there is less risk for performing a variety of illegal activities. This seriously leads to the Dark web .

Which should be investigated. But, one should even keep in mind that the inherent purpose of these activities are not to hurt the people, organizations, and societies.

Relatively than labelling an environment as unlawful and its users as 'different', a lot of understanding of the Dark web and its features and industrial structures is currently required.

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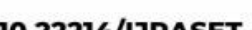
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Oral Cancer Detection Using Image Processing and Deep Neural Networks.

Vrushali Dilip Jadhav[1], Arati Maruti Jadhav[2], Asst.prof.S.V.Thorat[3]

[1]Vrushali Dilip Jadhav,MCA YTC Satara
[2]Arati Maruti Jadhav,MCA YTC Satara
[3]Asst.prof.S.V.Thorat, Dept. of MCA, Yashoda Technical Campus,Maharashtra,India

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Abstract-*The paper proposes accomplice revolutionary deep convolution neural community (DCNN) mixed with texture map for detection cancerous areas and staining the ROI for the duration of an unmarried version mechanically. The projected DCNN version carries 2 cooperative branches, specially accomplice better department to carry out carcinoma detection, and a decrease department to carry out linguistics segmentation and ROI marking. With the better department the community version extracts the cancerous areas, and additionally the decrease department makes the cancerous areas extra preciseness. To shape the alternatives inside the cancerous extra regular, the community version extracts the texture photos from the enter image. A window is then carried out to cipher the same old deviation values of the texture image. Finally, the excellent deviation values are accustomed assemble a texture map, that's partitioned into more than one patches and used due to the fact the laptop documents to the deep convolution community version. The tactic projected via way of means of this paper is called texture-map-primarily based totally department-collaborative community.*

Keywords— Deep Neural Network, Image Processing, Oral Cancer, Texture Map.

1.INTRODUCTION

Oral Cancer is especially denoted as class of head and neck cancer includes major sub regions of the lip covering mouth cavity, and tubular cavity (National Institutes of Health, 2018; WHO, 2017), consisting of concerning eighty fifth of the class. Right off the bat, carcinoma could be a life-threat sickness because of the very fact that its precursor symptoms and warning signs might not be ascertained by the patients routinely as a result of that this sickness could chop-chop progress into malignant neoclassic disease stage at intervals brief amount Oral cavity cancers also are better-known to own a high repetition rate compared to different cancers. Therefore, AN in-depth exploration of either its staging or its grading is important for its prognostic treatment. quite ninetieth of cancers that occur within the remoras square measure squalors cell carcinomas (SCC). This cancer cluster is characterized by animal tissue squalors tissue differentiation and aggressive growth disrupting the basement membrane of the inner cheek region. Commonly, clinical procedures for prognosis and treatment square measure evaluated on Tumor-Node-Metastasis (TNM) staging. However, a five-year survival report supported oral cancer reveals a prognosis rate of roughly thirty fifth to five hundredth guaranteeing quantitative microscopic anatomy grading of tumors, that comes with the in-depth study of assorted pathological aspects associated with SCC, as a additional advantageous

Method than growth staging for increasing malady survival rate. Hence, from a pathologist's point of read, providing precise histopathological identification within the context of multi-class grading is vital. This provides a principle to combat the problem by incorporating deep learning based malady identification or prediction strategies with clinical prospective that square measure hot analysis Oral SCC is

Morphologically classified into traditional, Well-differentiated, Moderately differentiated and poorly differentiated categories supported Brooder's system of microscopic anatomy grading. The cellular morphometry highlight the growth displays a terribly minute microscopic anatomy distinction separating the 3 categories that square measure very exhausting to capture by the human eye. it's remained elusive thanks to its extremely similar microscopic anatomy options that even pathologists realize troublesome to classify. Although most oral SCCs square measure moderately differentiated, all of them have totally different distribute characteristics and implicate different prognosis, repetition rate and survival, and treatment management.. Therefore, with the expansion of care standards everywhere the world, it's necessary for AN overhaul of pathology, which might involve additional fast and accurate identification.

2. LITERATURE SURVEY

Oral cancer is that the commonest form of head and neck cancer worldwide, with associate degree calculable 377,713 new cases and 177,757 deaths in 2020 [1]. Surgery is that the usual primary treatment and customarily yields high treatment success, with overall survival rates reaching 75–90% within the early stages [2, 3]. However, over hour of the cases are diagnosed at a sophisticated stage and progress with high morbidity and mortality [2,4]. Considering the terrible incidence and mortality rates, carcinoma screening has been a very important a part of several aid programs, as

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a live to enhance early detection of carcinoma [5]. Oral squalors cell cancer (OSCC) that makes up over ninetieth of carcinoma cases is usually preceded by oral doubtless malignant disorders (OPMD), like leukoplakia and erythroplakia [6]. The detection of OPMD, that encompasses a risk of malignant transformation, is of the utmost importance for reducing morbidity and mortality from carcinoma and has been the most focus of the screening programs [6]. However, the implementation of those programs, supported visual examination, has been found to be problematic during a real-world setting as they have faith in medical aid professionals, United Nations agency are usually not adequately trained or toughened to acknowledge these lesions [6,7]. The substantial heterogeneousness within the look of oral lesions makes their identification terribly difficult for aid professionals and is taken into account to be the leading explanation for delays in patient referrals to carcinoma specialists [7]. Besides, early-stage OSCC lesions and OPMD ar generally well and should seem as little, harmless lesions, resulting in late presentation of patients and ultimately resulting in additional diagnostic delay. Advances within the fields of pc vision and deep learning provide powerful ways to develop connected technologies that may perform an automatic screening of the oral fissure and supply feedback to aid professionals throughout patient examinations likewise on people for musing. The literature on image-based automatic designation of carcinoma has for the most part targeted on the utilization of special imaging technologies, like optical coherence picturing [8,9], hyper spectral imaging [10], and automotive vehicle light imaging [11–16]. On the opposite hand, there are some of studies performed with white-light photographic pictures [17–21], most of that target the identification of bound forms of oral lesions. The identification of OPMD is crucial for up early detection of carcinoma and so has a very important role within the development of carcinoma screening tools. during this study, our aim was to explore the potential applications of assorted pc vision techniques to the carcinoma domain within the scope of photographic pictures and investigate the prospects of a deep learning-based automatic system for carcinoma screening.

3 proposed system architecture

Training a deep convolution neural network (CNN) from scratch is tough as a result of it needs an outsized quantity of tagged coaching information and an excellent deal of experience to make sure proper convergence. A promising different is to fine-tune a CNN that has been pre-trained exploitation, for example, an outsized set of tagged natural pictures. However, the substantial variations between natural and medical pictures could advise against such knowledge transfer. We have a tendency to look for to answer the subsequent central question within the context of medical image analysis: will the use of pre-trained deep CNNs with spare fine-tuning eliminate the need for coaching a deep CNN from scratch? We have a tendency to thought-about four distinct medical imaging applications in three specialties (radiology, cardiology, and gastroenterology) involving classification, detection, and segmentation from three completely different imaging modalities, and investigated however the performance of deep CNNs trained from scratch compared with the pre-trained CNNs fine-tuned in a very layer-wise manner. Experiments consistently incontestable that the employment of a pre-trained CNN with adequate fine-tuning outperformed or, within the worst case, performed similarly as a CNN trained from scratch; fine-tuned CNN's were a lot of strong to the scale of coaching sets than CNNs trained from scratch; neither shallow standardization nor deep standardization was the optimum alternative for a selected application; and our layer-wise fine-tuning theme may supply a sensible thanks to reach the best performance for the applying at hand supported the amount of obtainable information.

Convolutional Neural Networks (CNN/ConvNet) can be excellent multi-layer neural networks aimed at mechanically extracting options directly from raw component images requiring little preprocessing. One of the ConvNet options is flexibility. From a small data set using pre-trained models like Image Net in the field of deep learning, we can say that the VGG design is the first deepCNN to achieve the most promising results. It's a very attractive network due to its simple and consistent design. VGG Internet is usually accustomed to confiscate the baseline option from a given input image. It works fine with the small dataset I created. Second, to balance the processing cost within ImageNet, the VGG design employs smaller convolution filters, reduces the types of receive array channels, and increases the depth of the network. The design of VGG16 mainly consists of three layers: convolution layer, pooling layer and absolute connection layer.Medical image classification plays an essential role in clinical treatment and teaching tasks. However, the traditional method has reached its ceiling on performance. Moreover, by using them, much time and effort need to be spent on extracting and selecting classification features. The deep neural network is an emerging machine learning method that has proven its potential for different classification tasks. Notably, the convolution neural network dominates with the best results on varying image classification tasks. However, medical image datasets are hard to collect because it needs a lot of professional expertise to label them these are linear support vector machine classifier with local rotation and orientation free features, transfer learning on two convolution neural network models.

Oral cancer is that the most typical head and neck cancer worldwide, inflicting more or less 177,757 deaths every year. carcinoma detected early are able to do survival rates of up to 75-90%. However, most cases ar diagnosed at a complicated stage. this is often primarily because of a scarcity of public awareness of carcinoma symptoms and delays in referrals to carcinoma specialists. Since early detection and treatment ar the foremost effective means that

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of up carcinoma outcomes, it's necessary to develop add-on vision technologies which will discover occult oral malignancies (OPMDs) that place patients in danger for cancer. , supply nice opportunities for carcinoma screening strategies. during this study, we have a tendency to investigated the potential application of pc vision techniques in carcinoma within the context of photographic pictures and explored the chance of an automatic system to discover OPMD.

Image segmentation is a crucial and tough a part of image process. it's become a hotspot within the field of image understanding. this is often additionally the bottleneck that limits the appliance of 3D reconstruction and alternative techniques. Image segmentation divides the complete image into multiple regions that share similar characteristics. Simply put, it serves to separate the target within the image from the background. Image segmentation strategies ar presently being developed in a very quicker and a lot of correct direction. By combining varied new theories and new techniques, we've got discovered a general segmentation formula which will be applied to differing kinds of pictures.

4.Feature Extraction

Feature plays a awfully vital role within the space of image process. Before obtaining options, varied image preprocessing techniques like binarization, thresholding, resizing, standardization etc. ar applied on the sampled image.

Feature extraction may be a methodology of capturing visual content of pictures for categorization and retrieval. Feature extraction is employed to denote a chunk of data that has relevancy for resolution the procedure task associated with bound application system. There ar 2 forms of texture feature measures. they're given as initial order and second order measures. within the initial order, texture measures ar statistics, calculated from a personal picture element and don't take into account picture element neighbor relationships.

5.Intensity Histogram

A oftentimes used approach for texture analysis is predicated on applied math properties of Intensity bar chart. A bar chart could be a applied math graph that permits the intensity distribution of the pixels of a picture, i.e. the amount of pixels for every glowing intensity, to be painted. By convention, a bar chart represents the intensity victimization X-coordinates going from the darkest (on the left) to lightest (on the right). Thus, the bar chart of a picture with 256 levels of gray are going to be painted by a graph having 256 values on the coordinate axis and also the variety of image pixels on the coordinate axis. The bar chart graph is built by reckoning the amount of pixels at every intensity worth

6.Conclusion:-

Here we have implemented the image processing and deep neural network technique for oral cancer detection till now we have implemented the image processing part and clustering techniques for the oral cancer images.

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Secure Desktop Computing In the Cloud

Priyanka Shankar Bhingardeve[1], Prajkta Mahendra Ghadge[2], Asst.Dr.S.P.Jadhav[3], Asst.prof.S.V.Thorat[4]

[1st]Priyanka Shankar Bhingardeve, MCA YTC, Satara
[2nd]Prajkta Mahendra Ghadge, MCA YTC Satara
[3rd]Dr.S.P.Jadhav,[4th]Prof.S.V.Thorat, Dept. of MCA Yashoda Technical Campus,Satara-415003

Abstract—

Computation that employees perform on their desktop and the management of the desktop computing infrastructure to the cloud, the need for securing such cloud-hosted user computing tasks and environments become paramount. In this paper, we present Venia, a secure cloud-based desktop computing platform designed to protect against both external and internal threats. Accessible to end-users through a thin Remote Desktop Protocol (RDP) client Venia isolates end-user's applications and data into containers and subjects the interactions with and among the containers to security policies. Following a principle of least privilege, Venia security policies control user's access to containers, network and file system interaction of the containers, cross-container data sharing and also enables collection of detailed logs for auditing purpose. Venia has been deployed to a 3rd party test environment where it demonstrated that end-users can perform the tasks they need on a daily basis, without introducing greater risk to the overall organization, and its currently undergoing security and performance evaluation by an independent evaluation team.

1. INTRODUCTION

The next step within the trend of moving backend services and supporting computing infrastructure to the cloud, is to maneuver end-user computing and its supporting infrastructure to the cloud additionally. Cloud computing provides economy of scale, eliminates the headache of computer code and hardware management and maintenance, and permits on-demand scaling and pay as you utilize rating. Properly architected, moving end-user computation to the cloud will offer a security profit. A conscientious cloud seller can offer stronger perimeter protection, specialised employees, and established tools, techniques and procedures for handling security incidents than a typical enterprise will generally deploy. However, sharing machine resources within the cloud presents a brand new set of security challenges for ensurinorganization and even worse, users from completely different organizations cannot breach security to attain malicious objectives.

2. Related Work

Secure Desktop computing in the cloud Current solutions for desktop computing within the cloud square measure based off of a Virtual Desktop Infrastructure (VDI) approach. VDI could be a variety of virtualization wherever entire desktop solutions are hosted within the cloud, so accessed employing a skinny consumer, usually with RDP. One such technology is Horizon seven by VMWare. in hand with these solutions is their wholesale exporting of the desktop atmosphere to the cloud. While helping to modify the digital geographic point and providing a centralized management over resource and network access, these solutions still maintain the appliance primarily based security problems inherent in a very ancient desktop.

3. Design Goals And Approach

The main style goals for Venia were:

• Role-specific UCEs: UCEs for individual users ought to consist of role-specific application bundles, wherever a job defines that desktop applications and resources area unit required to perform a particular job connected operate. A single user might have multiple roles, presumably requiring use of applications from multiple operative systems (e.g., Linux and Windows) in a very single role, and resources will be shared among completely different roles.

• Enterprise-specific security management and auditing:

Interaction between end-user skinny consumer and UCE ought to be encrypted. Resource access, data sharing and use of UCEs ought to be subject to enterprise-specific security controls and auditing policies.

• End-user expertise: End-user experience shouldn't change drastically from exploitation desktop atmosphere, in particular, end-users shouldn't need to re-authenticate themselves for role specific resource access, ought to realize familiar applications in their UCE, and be able to cut and paste wherever allowed by the enterprise policy.

• Administration: Venia ought to give a straightforward approach for administrators to outline enterprise-specific security and auditing policies, and a straightforward to outline role-specific application bundles and instantiating user-specific UCEs. To attain these goals, Venia was designed as distinct components a collection of microservices establishing the required enterprise IT services for a useful corporate infrastructure, a User cipher atmosphere (UCE) that defines the end-user space, together with their desktop, keep files and applications.

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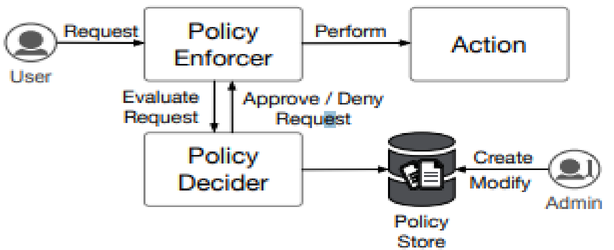

A. Enterprise IT Services as Microservices Enterprise IT services play a necessary and vital half in a corporate infrastructure. These services area unit liable for, among others, managing user access and authorization, and managing shared resources, like email servers, printers, and centralized filesystems. Venia contains a collection of microservices for performing aspects of those IT management functions, independent of the end-users space. Separating individual aspects of IT management responsibilities into distinct microservices that interoperate via a well-defined Representational State Transfer (REST) Application Programming Interface (API), and subjecting these interactions to strict security controls and auditing [6] reduces the chance of abusing the UCEs through the enterprise IT services, resulting in associate degree overall reduction of the attack surface of the UCEs.

Venia contains four microservices:

• **User Service:** provides the initial entry purpose into the Venia system, via a web-portal, and contains all of the business logic for authenticating a user against a directory service, like Active Directory, and obtaining all of their out there roles.

• **Virtue Service:** The Virtue Service coordinates communications between the opposite microservices, and is responsible for constructing the UCE. Once created, the only reference the UCE maintains back to the microservices is for coverage work events. This eliminates the potential for lateral attacks on the enterprise assets.

• **sensing element Service:** The sensing element service aggregates all of the logs across the Venia system. This centralized service provides the required observance and analysis of system activities.

• **Admin Service:** The admin service provides for the definition, management, and dissemination of policies

B. User Compute Environment (UCE)

UCE supports the acquainted daily interaction of the end-user to perform their daily tasks. The Venia UCE may be a single cloud based mostly machine instance that uses policy controlled containers to protect every Virtue, providing application isolation, and the ability to tightly management and monitor all actions and interactions. The UCE incorporates security mechanisms at multiple levels to ensure Associate in Nursing operational end-user expertise, whereas maintaining the goals and objectives of the policy.

4. Implementation

The current version of Venia is enforced as Associate in Nursing Amazon Web Services (AWS) application. This implementation consisted of two-subnets running in a very single Virtual Private Network (VPC). The sub-nets were divided between enterprise microservices in one, and UCEs in another. The only microservices that area unit accessible outside of the VPC area unit the Admin service, for policy construction, and also the Login service, for UCE creation.

A. UCE Implementation

Each Virtue lives through one LXC The display of every Virtue is shared to the host's X Server show to give a unified desktop look. The displays of every Virtue are shown within the sort of another window that identifies the containing Virtue. The Windows instance is connected throughRDP inside every Virtue on Associate in Nursing application basis. this permits Windows applications to own native support with the appearance of being on one seamless desktop among the Virtue. UNIX system applications area unit supported through the LXC containers the Virtues live to tell the tale. A writing board manager at the host level has been other to manage copy-paste options between the Virtue windows.

B. Demonstrative Examples

To verify our policy approach, we created and tested a few unique Virtues to exercise the capabilities of the system. Each of these Virtues were defined to address a specific security, or operational scenario.

5. Evaluation

To evaluate VENIA, we have a tendency to performed a series of performance overhead tests to estimate user perceptible overhead. For these many typical user operations, and compared against a regular desktop environment. every take a look at was conducted thrice, and the average was computed For these measurements, the quality desktop system was a VM on physically native hardware with four processor cores and 8GB of memory. VENIA was running on AWS t2.xlarge with four processors and 16GB of memory. To verify our policy approach, we have a tendency to created and tested many unique Virtues to exercise the capabilities of the system. Each of these Virtues were outlined to handle a particular security, or operational state of affairs. The automobile industry is investing in autonomous vehicles for driverless cars, which will have to analyze and make decisions on data that pertains to their surroundings for movements and directions. These vehicles need to transmit Data to the manufacturers so that they can track their usage and also get the required maintenance

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alerts. The data will be transmitted through networks resulting in congestion. To achieve low latency when accessing the network, it is necessary for the manufacturers to device new effective computing ways

6. CONCLUSION

As more front-end applications and computation continue to migrate to the cloud, the need for a secure and usable platform is paramount. With Venia, we have demonstrated an architecture for a secure cloud-based end-user computing solution. With this architecture, we were successful in separating enterprise IT functions from end-user tasks, which helped to reduce the amount of information available to an attacker while still providing an operable environment for the user. We further emonstrated that enterprise specific security controls and auditing requirements can be enforced on the UCEs, and provided an easy to use administrative toll to construct well defined policies for Virtues. Initial results show that running the applications in virtues within cloud-based UCEs subject to the applicable security controls and auditing policies do not drastically change the user's perception of the applications' response time, or constrain access to and use of information and resources they need to perform their job functions.

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Secure Cloud Computing

Harshada Shrikant Desai[1], Arati Sunil Kasurde[2], Dr.S.P. Jadhav[3], Asst.prof.S.S. Jadhav[4]

[1st]Harshada Shrikant Desai, MCA YTC, Satara
[2nd]Arati Sunil Kasurde, MCA YTC Satara
[3rd] Dr.S.P. Jadhav,[4] Prof.S.S. Jadhav Dept. of MCA Yashoda Technical Campus, Satara-415003

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ABSTRACT- It is nothing but way to deliver computing source in Cloud Computing. It ranges from data storage and processing to software i.e., customer relationship management systems, which is available instantly and in demand.

Manipulation, configuration and accessing is refered by Cloud Computing h/w & s/w source monitor. Online data storage, infrastructure and application is offered by it. In this research paper the researcher has discussed about the introduction part of the cloud computing, types of cloud computing. A research has also focus on Benefits limitation and future working of the cloud computing. In overall conceptual are discussion of cloud computing with the research has also focuses in future development.

1. INTRODUCTION

A social media is the depending with the cloud computing. cloud computing [i]simple line meaning is transport of the computing with the service.

Types of Cloud Computing are as follows:

- Infrastructure as a service (IaaS)
- Platform as a service (PaaS)
- Software as a service (SaaS)

Cloud computing can interacts with networks it's rules and regulations which is joined to its role. So, to start with the, we have already listen the asset of knowing to code for cloud computing.

1.1. What are Cloud Computing Security?

Collection of security measures are designed to secure cloud-based infrastructure, data and application. The other name of Cloud Computing security is collection of security measures. The above ways assure user and device verification. In today's era there is high demand to work in Cloud Computing field.

1.1. Use of Cloud Computing:

CC allows to the access file, resources, data, files whenever we are not connected to network the cloud computing is allows to access to their network with the help of internet.

1.2. Types of cloud Computing:

Commonly using the cloud computing use cases:

- IaaS
- PaaS
- SaaS

1] IaaS: IaaS is nothing but the h/w as a service. the access are in many clients and source internet used with online paying System. Iaas is using three types of the cloud first is the public second is private and third one is hybrid cloud. The cloud computing is used for helps to changing clients in the requirements and services.

2] PaaS: it is the purchase online mode for available in this platform. This platform as a service in cloud service provider. this is the developing the developer platform application. there are many languages is available in this like a java, php, perl and so on.

3]SaaS: this application stands for software as a service. This application is also known as the requirement for software. and also, customer with relationship is the management system making a electronic devices.

2. 7 CASES IN CLOUD COMPUTING:

1. Infrastructure as a Service (IaaS)
2. Platform as a Service (PaaS)
3. Software as a Service (SaaS)
4. Hybrid cloud and multi cloud
5. Test and development
6. Reach Robotics
7. Big data analytics.

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3. APPLICATION IN CLOUD COMPUTING AND DIIFERENT SECTORS:

i] Online data Storage:

The cloud computing is nothing but the accessing the various source file huge amount of data and files from with help of online data storage system.

Children love interacting with internet handling with them, and people are seeing excellent results in happiness and communication skills for children and students learning difficulties with the help of is videos and audios from devices.

ii] Backup and recovery:

The cloud computing is example of the data backup and restorage recover data in system with help of cloud computing.

For e.g.: WhatsApp, Instagram and so on likewise

iii] big data analysis: -

Most important part of application in cloud computing in network the huge amount of data or big data can be impossible to save or store in the system with help of cloud computing we can easily save our data in cloud computing.

iv] Testing and Development: -

The cloud computing is provided for easy and time consuming for the resources and also this is used in businesses and flexible services of business.

v] Antivirus Application: -

antivirus application is nothing but the monitoring to system virus and antivirus system such like as the malware, detects the security threats in the networking.

vi] E-commerce Application: -

e-commerce is the new technology to response quick opportunities to connecting with the e-commerce system.

vi] Cloud computing in Education:-

the cloud computing is the best way to learning virtually source for the student. There are many sources to learn educational things from various cloud computing platforms

Example: Children making android application to learn from the virtual applications like as zoom meeting, Vedantu app etc.

4. ADVANTAGES OF CLOUD COMPUTING

- Backup and restore data
- improve collaboration.
- Excellent Accessibility.
- Low maintenance cost
- Mobility
- Iservices in the paper use module
- Unlimited storage capacity.
- Data security

5. DISADVANTAGES OF SOCIAL CLOUD COMPUTING:

- internet connectivity.
- vendor lock-in
- limited control.
- security.

6. IMPORTANCE AND NEEDS OF CLOUD COMPUTING: -

- Cloud computing are enables with users to access system using a web browser regardless of location or what device they used for example: computer android mobiles are access via network. User can connect to the network from anywhere and any place.

  Cloud computing is a based service that can be meet in demand instantly.

7. IMPACTS OF CLOUD COMPUTING IN EVERYDAY LIFE:

i]Positive Impacts:

- the cloud computing is the changing our lives in many ways from social media shopping, streaming services and storing the files
- Positive impact in everyday life such as banking, email, media etc.
- It is committed for the described with directly to the technology.
- Cloud services also being with use of support is nothing but the impact.

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ii] Negative Impacts:

- i] Humans are depend on the fully socially in cloud computing and that case many loss faces in life.
- ii] Safe data is unsecure in the cloud computing platform. For example: Instagram, WhatsApp
- iii] Cloud like is the any other IT setup there are many problems and experience of technical with the problems faces. that can directly impact on damaging business e.g., reboots, network, downtime.

8. FUTURE CHALLENGES:

CC is mainly used and has become for popular latest in market. The cloud computing is nothing but the circulate on premises in computing to the internet.

i]security: there are the best concern in involving cloud computing service with the security.

ii]password security: many peoples access your account by vulnerable method, more important about your password security.

iii] cost management: cloud computing access in application to save or manage in your post and makes affordable.

iv] lack of expertise: lack of increasing the workload for the cloud technology increasing continuously improving their tools in technologies.

V] internet connectivity: the internet connectivity is part of cloud computing. the cloud services are depend on the internet connectivity, there are many network issues.

9. CONCLUSION:

Security is a big problem in CC. This needs to be overcome before services can be widely adopted. Some aspects of security are rarely in the field of technology, with external solutions, such as laws, regulations in human resource management and the like, it is important to find technological solutions to problems. It offers effective isolation between different clients while allowing resource sharing. Users can specify and manage their personal security and QoS policy settings just as they would traditional on-site management

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An Overview of Bluetooth Technology and its Communication Applications

Pooja Sunil Shevate[#1], Prerna Sambhaji Salunkhe[#2], Vanmala V. Kadam[*3] Pranjali S. Gade[*4]

[#]Student, MCA Department, Yashoda Technical Campus, Satara

[*]Asst. Prof. MCA Department, Yashoda Technical Campus, Satara

Abstract

Bluetooth is a new RF short-range wireless technology which is designed for wireless communication between different devices. There is increase in popularity of Bluetooth technology and is being accepted in today's world. There are organizations which are doing research on Bluetooth technology, but very few of their research analysis provide a balanced view of the technology, describing its implications for businesses, pros and cons. In this paper analysis have been done keeping in mind various perspectives of the Bluetooth technology. The analysis starts with a description of the technology in terms of its network infrastructure, hardware and software. Then it is continued by the Error corrections and retransmission. The analysis is done on macro analytical view including the business implications, advantages of this technology, its role in

Keywords: Bluetooth; Bluetooth architecture; Frequency-hopping spread spectrum (FHSS); Logical Link Control and Adaptation.

1. Introduction

We have all experienced the problem which arises when connections are made between peripheral and computer or connection between the electronicdevices. Thus the companies of telecommunications needed to develop an opened, low cost interface to make easier the communication between devices without using cables. Bluetooth is a wireless technology having very short range designed enabling communication between the devices like computers,entertainment systems and other electronic devices without the use of cables and connectors. There is a strong need for a better way for all the electronic devices to communicate with each other, in order to make the aforementioned systems, computers and/or Harald Blaotland (Bluetooth), a Danish king born in AD 908. The technology was developed by an Ericsson-led consortium, including Toshiba, IBM, Nokia and Intel. In early January 2000, the technology was further promoted by the Bluetooth Special Interest Group (SIG) comprised of 1371 member companies.

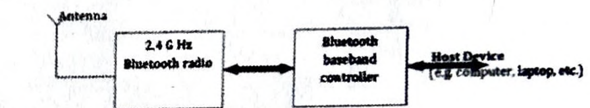
In this paper firstly we will discuss a microanalysis of Bluetooth Technology .The microanalysis of Bluetooth describes the technical details such as

2. Bluetooth Technology: A Microanalysis

Bluetooth is the technology which allows the devices to communicate with each other, synchronize data with each other, and connect to the Internet without the use of cables or wires. To add Bluetooth functionality to a computer or other host device a Bluetooth radio and base band controller can be installed on a device that

links to an integrated on a system board, a Universal Serial Bus (USB) port, or a PC Card. These components are shown in Fig. 1

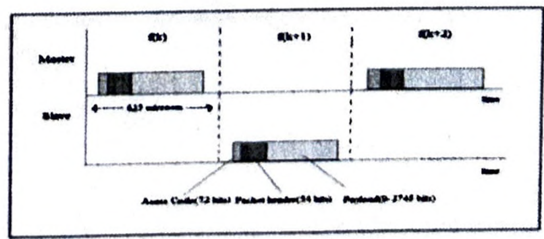
Fig.1 Bluetooth components



A. Technology overview of Bluetooth

In this section the technology specification have been explained .The Bluetooth technology is divided into two specifications: first is the core and second is the profile specifications. How the technology works is explained by the core specification, how to build interoperating devices using the core technologies is explained by the profile specification. Bluetooth air interface works on a antenna power of 0 dBm (1 mW) and be extended up to 20 dBm (100 mW) worldwide. This interface complies with ISM band rules up to 20 dBm in America, Japan, and most European countries. Frequency hopping method is used to spread the energy across the ISM spectrum in 79 hops displaced by 1 MHz, starting from 2.402 GHz and stopping at 2.480 GHz. The Bluetooth Special Interest Group is working to harmonize this 79- channel radio .These 79 channel radio are working globally and has initiated changes within Japan, Spain, and other countries. An electronic conversation determines whether they have data to share or whether one needs to control the other, whenever Bluetooth-capable devices come within range of one another.

Fig.2 Bluetooth Frame



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B. Network architecture

There is normally peer-to peer communications between Bluetooth devices in which each Bluetooth device is consider equal. In the network architecture of Bluetooth we use a term piconet that means two or more devices link into a small ad hoc network. In piconet one of the communicating device acts as the Master and the other devices act as slaves, this is for consider when the a piconet connection. The communicating devices are synchronized to the hopping sequence and master's clock in a piconet.

The Piconets can begin with two communicating devices, for example laptop and cell phone and may include maximum of eight devices. Users can automatically establish a connection with other Bluetooth device which is within its Bluetooth range. Bluetooth also allows automatic data synchronization among the communicating devices. In one piconet there can be only master device. This is because of the reason that Bluetooth technology supports both point-to- point and point-to-multipoint connections. Two or more piconets linked together forms a scatternet and one device in each piconet acts as a bridge between the two or more piconets formin a scatternet A scatternet. The piconets and scatternet is shown in Fig. 3. The radio or device is is assigned a 3 bit Active Member Address as soon as the device joins a piconet and thus allows other device on the piconet to address it and starts communication. The master must then take

C. Software architecture

Bluetooth devices use the Host Controller Interface (HCI) as a common interface between the Bluetooth host and the Bluetooth core in order to make different hardware implementations compatible.

Control Protocol (TCS). Segmentation and reassembly to allow larger data packets to be carried over a Bluetooth baseband connection issues are taken care by the Logical Link Control and Adaptation Protocol. The available services and their characteristics when, e.g. devices are moved or switched off are found out by SDP.

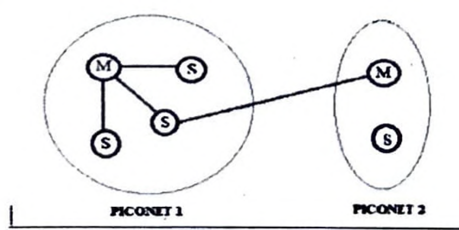

Fig.3 Piconets and Scatternet

The users having Bluetooth devices are connect to the other neighboring devices in a wireless manner via Service Discovery Protocol. Bluetooth wireless device users gets on-demand services is the main characteristics of the Service Discovery Protocol. The process that enables system developers to employ the Saluation architecture for service discovery in Bluetooth short-range radio frequency (RF) networks was defined by the Bluetooth Special Interest Group (SIG), in July, 2000. Moreover, new Bluetooth requirements, that comprises of Saluation and universal plug and play is being developed by SIG. This is described how to use other service discovery technologies.

D. Error corrections and retransmission

Forward error correction (FEC) and an automatic repeat request (ARQ) schemes for corrupted or missing data are the error correction schemes are usedby Bluetooth technology. The number of retransmitted data packets is reduced by the Forward error correction. The packets are flexible which permit the use of FEC. The FEC can be eliminated to reduce .

present in every packet in which link information is present. FEC protects the packet headers so that the bit errors can be survived. A 1-bit positive acknowledge (ACK) or negative acknowledge (NAK) is used by the Bluetooth ARQ scheme, indicating whether the data arriving at the receiving station matches the transmitted data. When there is no error in both the header error check and the cyclic redundancy check (CRC) then transmitting station gets an ACK. If there is error then transmitting station gats a NAK and retransmission of data takes place. Voice channels use an encoding scheme called continuous variable slope delta (CVSD) modulation that is immune to errors except in noisy environments. Voice transmissions are strictly real time, i.e. lost or damaged packets are neverretransmitted.

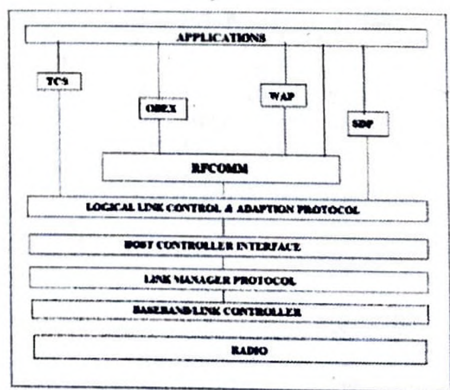

Fig.4 Bluetooth Protocol Stack

3. Bluetooth Technology: A Macroanalysis

Bluetooth is used in a wide range of mobile devices because of its versatile nature. Many of the Bluetooth-equipped devices are available today and and of the products based on Bluetooth are in progress. Many

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Bluetooth applications are described below. Bluetooth has a strong competition with other wireless connection devices like IrDA, Ultra Wideband Radio, etc. This segment discusses about the application of Bluetooth and comparison with other standards.

A. Bluetooth Applications

The earliest application of Bluetooth that became popular was wireless control of and communication between a mobile phone and a hands free set. Further application of bluetooth are Wireless control of and communication between a mobile phone and a Bluetooth compatible car stereo system, Wireless control of and communication with tablets and speakers such as iOS and Android devices and WirelessBluetooth headset and Intercom. Idiomatically, a headset is sometimes called a Bluetooth. Other application is Wireless streaming of audio to headphones without capabilities. Wireless networking

synchronizetheir shopping list with a current map of the store and get directions to each product in Bluetooth-networked stores. Hand-held computers can be used for making purchases by accessing Internet-based payment systems. With the help of Bluetooth technology, at the business centre, hotel guests could more easily use equipment such as printers. Bluetooth could allow for low-cost voice calls in an exclusive facility, such as a frequent-flier lounge at an airport. The airline could give the customers special rates on calls from Bluetooth handsets.

B. Comparison with Other Technology

Bluetooth application could allow cardiac patients being monitored through their mobile phones. Instead of being confined to local area networks within the hospital, patients can be anywhere within the range of their Bluetooth compatible mobile phones. A Bluetooth device could turn picks up signals directly from the patient's heart monitor and convey it to the mobile phone and relays the information to the monitoring system present in the hospital. The complex and tedious task of networking between the computing devices yet have the power of connected devices is done by Bluetooth network installed in the office. Workers can connect to the network anywhere within the office.

All of peripherals of the office are connected in a wireless manner. PCs or notebooks connections can be made without troublesome cable attachments to the printers, scanners and faxes. Any selected documents and electronic business cards can be instantlyexchanged with selected participants through Bluetooth in meetings and conferences. Local Bluetooth connections also facilitate E-mail, Internet, and Intranet access. A Bluetooth PDA or notebook can

notebook computers of all listeners so thatthe listeners will be able to follow the presentation on their own computers.

Technology	Comparison with bluetooth	
	Advantages	Disadvantages
IrDA	Reliable Inexpensive Higher Capacity	Line of Sight Compatibility One to One Only
Cable synchronizing	Low Interference Higher capacity	Needs special hardware One-to-one only
Wireless LAN	Higher capacity Larger number of simultaneous users	Bulkier hardware More expensive Higher power consumption Compatibility
Home RF	High user per net	Reliability Security
UWB Radio	Higher Capacity Low Power Consumption	Complex Size not known

C. *Bluetooth: Advantages*

There are three important features of Bluetooth from the user's point of view. Firstly , the user does not have to worry about the cables to attach all components as the technology is wireless technology. Bluetooth is inexpensive, it is the second feature of technology. By the end of 2002, it should only add $5 instead of $15. Third and the last, it is simple to use. Without any user

input the devices find one another and starts communicating. Bluetooth has made cellular telephones hands-free less susceptible to regulation for use in automobiles in the field of cellular industry. Bluetooth headset has protected the user's brain from the higher levels of RF radiation emitted by the cellulartelephones. Bluetooth has also helped the manufacturers of other portable devices. Such as ,it is difficult to keep the electronic devices having database synchronized . With Bluetooth, anytime one of these devices comes into proximity with another, they will communicate and synchronize their databases to make sure that both devices contain the most recent information.

4. The future of Bluetooth

There will be worldwide implementation of Bluetooth technology, in the near future. Workplace can be influenced in the following ways: More efficient configuration of workplace because there will be less use of cables and wires. Workers will have embedded in their ID badge a chip that will automatically log in /out their computers .The same badge will give them

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access to copy machines, fax machines, computer terminals, etc., throughout building. If the worker is not on the desk, phone calls can be rerouted them. Computer schedule, documents, e-mail, and all other information can seamlessly be synchronized. In late June 2000, Troy XCD and InTechnology announced a partnership that would enable

Conclusion

Bluetooth technology is very important technology that can make communication within the range between the devices possible without the use of wires. Bluetooth is being used in blurring the boundaries between home, the office, and the outside world. Bluetooth promises a seamless connectivity which makes it possible to explore a range of interactive and highly transparent personalized services. However, there are some issues to be sorted out. One potential problem being faced by Bluetooth is that hardware for it is being created faster than software. A report by Aegis Systems says that Bluetooth, other wireless networking systems, microwave ovens, outdoor broadcast units, and radio-based CCTV units may all interfere with each other.

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Kajal Kadam[1], Aliya Shaikh[2], Asst.Prof. P.S.Gade[3]

[1]*Kajal Kadam, YTC, Satara, Maharastra India*
[2]*Aliya Shaikh, YTC, Satara Maharastra India*
[3]*Asst.Prof.P.S.Gade,YTC, Satara, Maharastra India*

Abstract - *The likelihood of a security breach has never been higher due to the variety of new threats that are developing from both inside and outside your network. The combination of all these risk variables with a significant human component presupposes that everything has been set up and configured correctly to get the best results possible from each security instrument. In order to address this issue, organisations often spend more money on security controls, which increases management visibility and makes it easier for team like Security operation to see better results and provide good return on investment.*

The author covers the current trends in cybersecurity in this research report. Investigations have also focused on cybersecurity trends advantages and potential growth. The conceptual analysis of trends as a whole in the research has also given consideration to future evolution.

Key Words: Cyber Security, Data, Attacks, Threats, Cryptocurrency, Breach

1. INTRODUCTION

The cyber security domain growing faster as both offensive and defensive security service providers compete to outwit one another. Technology is constantly developing and improving, and new threats and creative solutions to combat them are constantly emerging. As more people started working from home recently due to the pandemic, fraudsters discovered new techniques, tactics, and strategies to take over networks and steal data in order to exact a ransom.

The newest developments in cyber security are discussed in this overview.

2. CYBER SECURITY TRENDS

2.1 Machine Learning

Although it is one of the newest technologies in cybersecurity, machine learning is playing a bigger and more proactive role. One of the reasons is that machine learning makes cybersecurity easier, more efficient, and less expensive (ML). This system builds patterns and manipulates them, anticipating and responding to active attacks in real-time using sophisticated algorithms that are based on complex data. To put it another way, applying ML to cybersecurity systems helps them to assess threat patterns and learn the habits of hackers, assisting in the prevention of future attacks and decreasing the amount of time cybersecurity experts must dedicate to repetitive operations.

ML makes cybersecurity easier to use, more efficient, and more affordable all at once. ML creates patterns and uses algorithms to alter them from a complex set of data. It can therefore anticipate dangers in real time and respond to them accordingly. In order to create efficient algorithms, this technology primarily depends on complex and rich data. The information must come from everywhere and must cover as many probable outcomes as is practical. In order to assess attack trends and learn hackers' tactics, cybersecurity systems can now use machine learning (ML)

2.2. Artificial Intelligence (AI)

It is impossible for human being to operate large number of cyber security threats. As a result, organisations are increasingly turning to AI and ML to hone their security infrastructure. Al has played a key role in developing automatic threat detection, face recognition, natural language processing, and security automation systems. Al also enables the much quicker analysis of enormous amounts of danger data. This is advantageous for both huge enterprises dealing with massive amounts of data and small or mid-sized businesses with sometimes under-resourced security teams. While Al has enormous potential for businesses to detect threats more thoroughly, thieves are also leveraging the technology to automate their attacks by using model-stealing and data-poisoning methods.

2.3 Need of Multi-Factor Authentication

Password protection is no longer enough due to the sophistication of modern cyberattacks. Compared to a basic password, a multi-factor authentication (MFA) is much more secure. By simply adding an additional layer of security, multi-factor authentication helps to prevent illegal access to online accounts. MFA makes sure that businesses can better safeguard employee data and manage access. Every time someone signs in, they must additionally provide a verification code that is sent to their registered phone number or through an authenticator app. The "gold standard" of authentication is multi-factor authentication (MFA).

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In order to solve this problem, organisations will increasingly use on application-based MFA tools like Microsoft Authenticator, Google Authenticator, One Span Authenticator, and others. Despite this, because SMS and voice MFAs are not end-to-end encrypted, they are still susceptible to attacks. Microsoft also suggested their customers to use app-based authenticator with the keys instead of phone-based authenticator. Despite this SMS and voice MFA continue to be vulnerable as these channels are not encrypted.

2.4 Rise of Ransomware

Even though ransomware has been a threat for over 20 years, it is still becoming worse. The frequency of ransomware cyberattacks is considered to have significantly grown now a days. In this cyberattack attacker finds access to the sensitive data of a person, a group, or a organization and then encrypt it so they cannot access it. They then convey a threat to reveal personal information if a ransom is not paid, which is typically made in bitcoin.

The burden of this cyber threat is significant given the sensitive data at stake as well economic impact of paying the Ransome. Remote working and the growing digitization of many enterprises have given ransomware new targets.

As the number of attacks is increased, the quantity of Ransome paid is also increased. Most of the time, hackers demand such payments in obscure cryptocurrency.

5. Breach and Attack Simulation (BAS)

In the last two decades, cyberattacks have undergone a significant evolution in terms of their capabilities, reach, consequences, and variety of targets. Cybercrime is causing record-breaking losses worldwide, and it appears that this trend will continue. Security managers and executives are aiming to improve their company's security posture because of the increased danger of attacks. BAS technologies are described as technology "that enable enterprises to continuously and consistently simulate the full attack cycle against enterprise infrastructure, using software agents, virtual machines, and other means, including insider threats, lateral movement, and data exfiltration."

The uniqueness of BAS resides in its capability to deliver consistent and reliable simulation assessment with minimal risk, as well as its use in warning IT and business stakeholders about existing security posture gaps or confirming that security infrastructure, configuration settings, and detection technologies are functioning as intended. When utilised in addition to red team or penetration testing assessment, BAS can help validate to identify whether security operations and the SOC staff can detect certain cyberattacks.

2.6. Cloud Security

More and more businesses are moving to the cloud as a result of the significant benefits it provides. To combat online criminals, a cutting-edge predictive security model must be used if the cloud is to be secure. Attacks on cloud services have grown over the past ten years, making them a risky way to store or transfer sensitive data. Secure encryption, authentication, and audit logging are not typically provided by cloud services. Others do not properly separate user data from that of other cloud tenants who share the same space. Security experts believe that cloud security needs to be strengthened as a result.

Threats can now be detected by predictive security before an attacker even makes a move. It has the ability to identify attacks that get past other endpoint security. In order to strengthen security, businesses are implementing predictive security clouds, and some industries have also turned to multi-factor authentication.

Although cloud computing has many advantages, including cost-effectiveness, scalability, and efficiency, it also has drawbacks that cannot be avoided. They are a top target for attackers as well. Insecure interfaces, account theft, and data breaches are all frequently brought on by improperly configured cloud settings.

2.7 IOT

The Internet of Things (loT) has completely changed the way we interact with devices. Despite some security issues with loT devices, which are common, most consumers have a high level of confidence in them. loT devices are currently dominating the consumer markets. Smart gadgets, air conditioning with built-in intelligence, wearable fitness trackers, and voice assistants like Google Home and Amazon Echo are a few examples of loT devices. Despite the fact that loT provide more convenience, they also pose greater risks to a user's data. In the event that a device is compromised or taken over, it could essentially act as a listening device and steal data from the network.

Hackers have discovered a new entry point for information and are making the most of it. To gain access to security systems, for instance, hackers frequently attempt to hack into connected camera networks or devices. If proper security measures aren't taken, the network is also exposed to software bugs or vulnerabilities by the communication protocols used to connect with various devices, increasing the possibility of outbreaks.

3. ADVANTAGES

- Unauthorized access to data and network is protected.

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- Early detection of threats and vulnerabilities.
- Protection from cyber-attacks/breaches.

4. CONCLUSION

With new and complex risks emerging every day, the cyberworld is continually evolving. Although most people think they are protected from cyber threats, almost everyone is actually vulnerable. There are always new strategies to defend your firm against these threats as new trends emerge. Keeping an eye out for any new cybersecurity trends you can ensure the security of your organisation. There are numerous new trends that you need carefully investigate in order to choose the one that best suits your demands.

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An Overview of Bluetooth Technology and its Communication Applications

Pooja Sunil Shevate[#1], Prerna Sambhaji Salunkhe[#2], Vanmala V. Kadam[*3] Pranjali S. Gade[*4]

[#]*Student, MCA Department, Yashoda Technical Campus, Satara*

[*]*Asst. Prof. MCA Department, Yashoda Technical Campus, Satara*

Abstract

Bluetooth is a new RF short-range wireless technology which is designed for wireless communication between different devices. There is increase in popularity of Bluetooth technology and is being accepted in today's world. There are organizations which are doing research on Bluetooth technology, but very few of their research analysis provide a balanced view of the technology, describing its implications for businesses, pros and cons. In this paper analysis have been done keeping in mind various perspectives of the Bluetooth technology. The analysis starts with a description of the technology in terms of its network infrastructure, hardware and software. Then it is continued by the Error corrections and retransmission. The analysis is done on macro analytical view including the business implications, advantages of this technology, its role in

Keywords: Bluetooth; Bluetooth architecture; Frequency-hopping spread spectrum (FHSS); Logical Link Control and Adaptation.

1. Introduction

We have all experienced the problem which arises when connections are made between peripheral and computer or connection between the electronicdevices. Thus the companies of telecommunications needed to develop an opened, low cost interface to make easier the communication between devices without using cables. Bluetooth is a wireless technology having very short range designed enabling communication between the devices like computers,entertainment systems and other electronic devices without the use of cables and connectors. There is a strong need for a better way for all the electronic devices to communicate with each other, in order to make the aforementioned systems, computers and/or Harald Blaotland (Bluetooth), a Danish king born in AD 908. The technology was developed by an Ericsson-led consortium, including Toshiba, IBM, Nokia and Intel. In early January 2000, the technology was further promoted by the Bluetooth Special Interest Group (SIG) comprised of 1371 member companies.

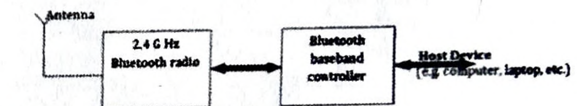
In this paper firstly we will discuss a microanalysis of Bluetooth Technology .The microanalysis of Bluetooth describes the technical details such as

2. Bluetooth Technology: A Microanalysis

Bluetooth is the technology which allows the devices to communicate with each other, synchronize data with each other, and connect to the Internet without the use of cables or wires. To add Bluetooth functionality to a computer or other host device a Bluetooth radio and base band controller can be installed on a device that

links to an integrated on a system board, a Universal Serial Bus (USB) port, or a PC Card. These components are shown in Fig. 1

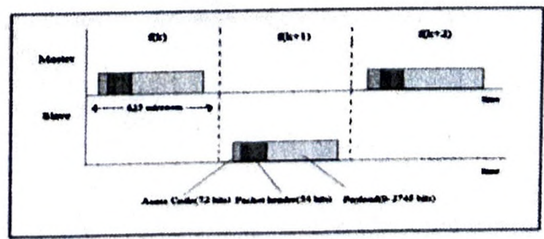
Fig.1 Bluetooth components



A. Technology overview of Bluetooth

In this section the technology specification have been explained .The Bluetooth technology is divided into two specifications: first is the core and second is the profile specifications. How the technology works is explained by the core specification, how to build interoperating devices using the core technologies is explained by the profile specification. Bluetooth air interface works on a antenna power of 0 dBm (1 mW) and be extended up to 20 dBm (100 mW) worldwide. This interface complies with ISM band rules up to 20 dBm in America, Japan, and most European countries. Frequency hopping method is used to spread the energy across the ISM spectrum in 79 hops displaced by 1 MHz, starting from 2.402 GHz and stopping at 2.480 GHz. The Bluetooth Special Interest Group is working to harmonize this 79- channel radio .These 79 channel radio are working globally and has initiated changes within Japan, Spain, and other countries. An electronic conversation determines whether they have data to share or whether one needs to control the other, whenever Bluetooth-capable devices come within range of one another.

Fig.2 Bluetooth Frame



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B. Network architecture

There is normally peer-to peer communications between Bluetooth devices in which each Bluetooth device is consider equal. In the network architecture of Bluetooth we use a term piconet that means two or more devices link into a small ad hoc network. In piconet one of the communicating device acts as the Master and the other devices act as slaves, this is for consider when the a piconet connection. The communicating devices are synchronized to the hopping sequence and master's clock in a piconet.

The Piconets can begin with two communicating devices, for example laptop and cell phone and may include maximum of eight devices. Users can automatically establish a connection with other Bluetooth device which is within its Bluetooth range. Bluetooth also allows automatic data synchronization among the communicating devices. In one piconet there can be only master device. This is because of the reason that Bluetooth technology supports both point-to- point and point-to-multipoint connections. Two or more piconets linked together forms a scatternet and one device in each piconet acts as a bridge between the two or more piconets formin a scatternet A scatternet. The piconets and scatternet is shown in Fig. 3. The radio or device is is assigned a 3 bit Active Member Address as soon as the device joins a piconet and thus allows other device on the piconet to address it and starts communication. The master must then take

C. Software architecture

Bluetooth devices use the Host Controller Interface (HCI) as a common interface between the Bluetooth host and the Bluetooth core in order to make different hardware implementations compatible.

Control Protocol (TCS). Segmentation and reassembly to allow larger data packets to be carried over a Bluetooth baseband connection issues are taken care by the Logical Link Control and Adaptation Protocol. The available services and their characteristics when, e.g. devices are moved or switched off are found out by SDP.

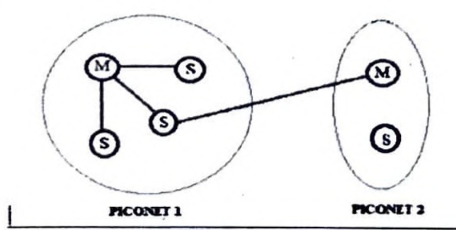

Fig.3 Piconets and Scatternet

The users having Bluetooth devices are connect to the other neighboring devices in a wireless manner via Service Discovery Protocol. Bluetooth wireless device users gets on-demand services is the main characteristics of the Service Discovery Protocol. The process that enables system developers to employ the Saluation architecture for service discovery in Bluetooth short-range radio frequency (RF) networks was defined by the Bluetooth Special Interest Group (SIG), in July, 2000. Moreover, new Bluetooth requirements, that comprises of Saluation and universal plug and play is being developed by SIG. This is described how to use other service discovery technologies.

D. Error corrections and retransmission

Forward error correction (FEC) and an automatic repeat request (ARQ) schemes for corrupted or missing data are the error correction schemes are usedby Bluetooth technology. The number of retransmitted data packets is reduced by the Forward error correction. The packets are flexible which permit the use of FEC. The FEC can be eliminated to reduce .

present in every packet in which link information is present. FEC protects the packet headers so that the bit errors can be survived. A 1-bit positive acknowledge (ACK) or negative acknowledge (NAK) is used by the Bluetooth ARQ scheme, indicating whether the data arriving at the receiving station matches the transmitted data. When there is no error in both the header error check and the cyclic redundancy check (CRC) then transmitting station gets an ACK. If there is error then transmitting station gats a NAK and retransmission of data takes place. Voice channels use an encoding scheme called continuous variable slope delta (CVSD) modulation that is immune to errors except in noisy environments. Voice transmissions are strictly real time, i.e. lost or damaged packets are neverretransmitted.

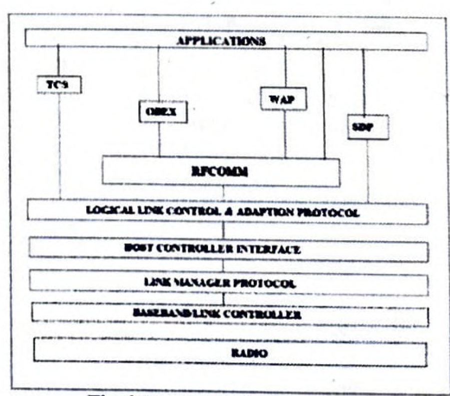

Fig.4 Bluetooth Protocol Stack

3. Bluetooth Technology: A Macroanalysis

Bluetooth is used in a wide range of mobile devices because of its versatile nature. Many of the Bluetooth-equipped devices are available today and and of the products based on Bluetooth are in progress. Many

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Bluetooth applications are described below. Bluetooth has a strong competition with other wireless connection devices like IrDA, Ultra Wideband Radio, etc. This segment discusses about the application of Bluetooth and comparison with other standards.

A. Bluetooth Applications

The earliest application of Bluetooth that became popular was wireless control of and communication between a mobile phone and a hands free set. Further application of bluetooth are Wireless control of and communication between a mobile phone and a Bluetooth compatible car stereo system, Wireless control of and communication with tablets and speakers such as iOS and Android devices and WirelessBluetooth headset and Intercom. Idiomatically, a headset is sometimes called a Bluetooth. Other application is Wireless streaming of audio to headphones without capabilities. Wireless networking

synchronizetheir shopping list with a current map of the store and get directions to each product in Bluetooth-networked stores. Hand-held computers can be used for making purchases by accessing Internet-based payment systems. With the help of Bluetooth technology, at the business centre, hotel guests could more easily use equipment such as printers. Bluetooth could allow for low-cost voice calls in an exclusive facility, such as a frequent-flier lounge at an airport. The airline could give the customers special rates on calls from Bluetooth handsets.

B. Comparison with Other Technology

Bluetooth application could allow cardiac patients being monitored through their mobile phones. Instead of being confined to local area networks within the hospital, patients can be anywhere within the range of their Bluetooth compatible mobile phones. A Bluetooth device could turn picks up signals directly from the patient's heart monitor and convey it to the mobile phone and relays the information to the monitoring system present in the hospital. The complex and tedious task of networking between the computing devices yet have the power of connected devices is done by Bluetooth network installed in the office. Workers can connect to the network anywhere within the office.

All of peripherals of the office are connected in a wireless manner. PCs or notebooks connections can be made without troublesome cable attachments to the printers, scanners and faxes. Any selected documents and electronic business cards can be instantlyexchanged with selected participants through Bluetooth in meetings and conferences. Local Bluetooth connections also facilitate E-mail, Internet, and Intranet access. A Bluetooth PDA or notebook can

notebook computers of all listeners so thatthe listeners will be able to follow the presentation on their own computers.

Technology	Comparison with bluetooth	
	Advantages	Disadvantages
IrDA	Reliable Inexpensive Higher Capacity	Line of Sight Compatibility One to One Only
Cable synchronizing	Low Interference Higher capacity	Needs special hardware One-to-one only
Wireless LAN	Higher capacity Larger number of simultaneous users	Bulkier hardware More expensive Higher power consumption Compatibility
Home RF	High user per net	Reliability Security
UWB Radio	Higher Capacity Low Power Consumption	Complex Size not known

C. *Bluetooth: Advantages*

There are three important features of Bluetooth from the user's point of view. Firstly , the user does not have to worry about the cables to attach all components as the technology is wireless technology. Bluetooth is inexpensive, it is the second feature of technology. By the end of 2002, it should only add $5 instead of $15. Third and the last, it is simple to use. Without any user

input the devices find one another and starts communicating. Bluetooth has made cellular telephones hands-free less susceptible to regulation for use in automobiles in the field of cellular industry. Bluetooth headset has protected the user's brain from the higher levels of RF radiation emitted by the cellulartelephones. Bluetooth has also helped the manufacturers of other portable devices. Such as ,it is difficult to keep the electronic devices having database synchronized . With Bluetooth, anytime one of these devices comes into proximity with another, they will communicate and synchronize their databases to make sure that both devices contain the most recent information.

4. The future of Bluetooth

There will be worldwide implementation of Bluetooth technology, in the near future. Workplace can be influenced in the following ways: More efficient configuration of workplace because there will be less use of cables and wires. Workers will have embedded in their ID badge a chip that will automatically log in /out their computers .The same badge will give them

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access to copy machines, fax machines, computer terminals, etc., throughout building. If the worker is not on the desk, phone calls can be rerouted them. Computer schedule, documents, e-mail, and all other information can seamlessly be synchronized. In late June 2000, Troy XCD and InTechnology announced a partnership that would enable

Conclusion

Bluetooth technology is very important technology that can make communication within the range between the devices possible without the use of wires. Bluetooth is being used in blurring the boundaries between home, the office, and the outside world. Bluetooth promises a seamless connectivity which makes it possible to explore a range of interactive and highly transparent personalized services. However, there are some issues to be sorted out. One potential problem being faced by Bluetooth is that hardware for it is being created faster than software. A report by Aegis Systems says that Bluetooth, other wireless networking systems, microwave ovens, outdoor broadcast units, and radio-based CCTV units may all interfere with each other.

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Security Factors affecting Internet of Things

Ajay Jadhav[1], Sagar Khot[2], Sana Bagwan[3], Vanmala Kadam[4], Prof. Shweta Thorat[5]
[1, 2, 3]MCA Department, Shivaji University, Kolhapur
[4]Associate Prof. MCA Department, [5]Professor, Yashoda Technical Campus, Satara

Abstract: Internet of things (IoT) is the following huge thing in the networking field. The vision of IoT is to connect day by day used gadgets that have the capacity of sensing and actuation to the internet. This can or may additionally or might not contain human. In this paper we are able to go through all of the demanding situations of IOT and mainly focus on IOT safety undertaking. IoT entails adding net connectivity to a system of interrelated computing gadgets, mechanical and digital machines, items, animals and/or people. Each "component" is furnished a completely unique identifier and the capability to automatically switch statistics over a community. Allowing devices to connect with the internet opens them as much as some of severe vulnerabilities if they're now not nicely included.
Keywords: Internet of things, security challenges

I. INTRODUCTION

Internet of Things (IoT) is extra than the machine to machine conversation. “IoT is a network of dedicated bodily gadgets (things) that comprise embedded generation to experience or interact with their internal state or external environment. The IoT accommodates an environment that consists of things, conversation, packages and records evaluation. Massive objects are to be connected to internet.

The devices will interact with different devices by way of pervasive computing but there's heterogeneity within the architectures. On pinnacle of this protection is any other massive project in IoT implementation. Primary goal of IoT is to reduce strength consumption and decrease the usage of resources.

A. Ease of Use

IoT discovers application in numerous fields like medicinal drug e.g. looking at heartbeat tempo of patient and tracking the records and with data it's going to decide or ship the data to specialist about it, domestic robotization for instance controlling room temperature, business organizations as an instance quality control, fitness hardware as an example energy to be scorched, smart city regions as an example transport on course sign to daily workers and so forth. Remote sensor systems which are meanings of IoT can show to us a few preparations.

Far off sensor structures is utilized to hit upon the object and transmit the facts, for detecting it need not hassle with an awful lot calculation control but transmitting the detected data desires some correspondence way which may additionally activate protection trouble.

In this paper, we examine of the principle IoT security threats, consisting of clever cars, clever domestic, aircraft, and provides considerations to network standards for the IoT, and advise future studies consideration to receive a at ease IoT offerings.

II. LITERATURE REVIEW

Valeriy G. Semin Russian State Social University Moscow, Russia , Eugeniy R. Khakimullin Academy of State fire service of EMERCOM of Russia Moscow, Russia mentioned Filling the idea of "Internet of factors" with a result of technological content material and implementation of sensible solutions, starting from 2010, is taken into consideration a solid fashion in facts technologies, mostly due to the good sized distribution of wi-fi networks, the emergence of cloud computing, the development of intermachine interaction technology, the transition to IPv6 and software program improvement -configurable networks.

1) Endless sharing of data among “things” plus the customers can increase when inappropriate verification, validation and permission. Presently, there are certainly not any dependable platforms that deliver entry to manipulate and personalized safety policy based entirely on operator’s requirements and context across one of a kind styles of “things”. The“things” in any IoT network are regularly ignored and overlooked; consequently, they are at risk of outbreaks. Furthermore, maximum IoT network and the communications make spying easy as the network are wireless. The destiny considerable for implementation of IoT will

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increase the facts security threats some distance additionally broad than the net has till now. Within any ad-hoc IoT network, infrastructure isn't always required where IoT nodules are restricted and self-organised, community. Hence security of such IoT nodules which execute in such ad-hoc networks are increasingly turning into an important and vital undertaking to clear up countless requests. And so such ad-hoc IoT network will become marketably possible. As ad-hoc IoT community has a regularly changing network topology, and the IoT nodes have restricted processor energy, reminiscence length and battery energy, a centralised safety authentication server/node will become impractical to be applied.

2) The knowledge of security algorithm performances via reading the overall performance of numerous algorithms that may be implemented on IoT devices; making use of some of safety algorithms, as an example, authenticated encryption schemes, AES, block ciphers message authentication codes, hash features, elliptic curves and so forth. to compare and examine their performances on an IoT platform, Raspberry Pi, an embedded gadget which is known as the black field and used as an IoT device in may fit.
3) The Internet of Things market is growing rapidly, but there are also a lot of issues that are following this expansion the security of these gadgets isn't constantly where it need to be, which means that, with the anticipated persisted growth of the tech, there may be a few big news objects or issues growing. For a ability look at what a number of those information items would possibly appear like, contributors of the younger Entrepreneur Council, below, speak areas of boom for IoT devices, in addition to discuss in which trouble may be brewing.

a) Automotive Iot
b) More Secuirty Targets
c) Hardware and firewalls becoming more prevalent
d) New and more expansive solutions
e) Cyber security for Smart homes
f) Data policies and disclosures
g) Hacking with Facial Recognition

III.RESEARCH METHODOLOGY

IoT builders should include safety on the start of any customer-, enterprise- or commercial-based device improvement. permitting security by default is vital, as well as presenting the most latest running systems and the usage of cozy hardware.

Hardcoded credentials need to by no means be a part of the layout manner. a further degree builders can take is to require credentials be updated through a person earlier than the tool capabilities. If a tool comes with default credentials, users ought to update them the usage of a robust password or multifactor authentication or biometrics in which possible.

We recently introduced in an IETF draft ("Blockchain transaction Protocol for Constraint Nodes") the BIoT paradigm, whose main plan is to insert sensing element knowledge in blockchain transactions. as a result of objects don't seem to be logically connected to blockchain platforms, controller entities forward all data required for dealing forgery. never less so as to get cryptological signatures, object wants some sure computing resources.

*While executing technology the "Internet of things" inherets in customary communication networks which adds to the privacy violations: repeat, spying, data misrepresentation, etc. Presently, issues to secure the user data, significantly personal data. The DDoS attack in 2016, regularly recognized and high-profile are associated to the technology of "Internet of things". For instance, there was a widespread network of nearly 152 000 camera had "hit" on the French provider while creating an ultimate load up to 1.5 TB/s.

It should be observed that the makers decline to debate on what was happening, and we need to perceive them. Precisely implement the promotion of an enormous variety of tools which terribly costly. The buyer is more concerned with the worth and not with the fore coming danger and risks, so, and even the market place is obsessed to demand.

Thus most issues of the technology of "Internet of things", arises due to lack of reading of data safety techniques that affects the integrity.

The difference in impressions of the wrong individual is kind of wide: inoffensive deception to prohibited activities. For instance, a hacker has changed communicated to the pacer data by dynamical during its process. Or, as an example, incorporated Associate in Nursing empty kettle.

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Present obtainable info on the triad it security infrastructure of the web of things in an exceedingly systematic means (see table).

The security requirement.	The status quo.	Consequences.	Solutions.
Privacy policy	1.Manufacture is do not take into account the security because of increasing prices of devices and reduce competitivene 2. Users do not understand the reality of the many threats. 3. The problem together-go data usage.	1. Misuse of personal data, including marketing research	1. Outreach on security issues. 2..Implementation of standards for technology. 3. The use of a protected architectures, including the introducetion.
Integrity	1. The possibility of changes for misrepresent-tation or illegal action	1. Deception of the user. 2. Causing harm or damage to a user	1. Implementati-on of standards for technology. 2. The use of a protected archi-tectures.
Availability	1. The possi-bility of DDoS attacks on the device. 2. The use of devices as bots for DDoS attacks.	1. The malfunction of the system. 2. The use of devices of unlawful acts.	1. Implementati-on of technology standards. 2. Identification and assessment of all devices, especially sensitive to cracking. 3. The use of contributed funds to counter DDoS attacks.

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Additional focus should be given to the question of security systems structure. "Internet of things" constructed on "weak points". While someone form a system of organized components continuously have an opinion of interaction over which records are traded amongst various parts of the network: as per many professionals, they drive more attention on protecting such facts. "Here there may arise ample of intermediate facts on the data trail, wherein they can be diverted.
It is necessary to inform additionally regarding the matter of certification. the majority the technologies of BCI developed and enforced foreign suppliers. Certified (tested) means that, appropriate to be used in BCI, the overwhelming majority of cases there's not. Recall that certification, as a rule, is performed in accordance with antecedently approved necessities. Thus, there's a requirement for a system of instruction of innovative tools, IOT and periodic change of the restrictive framework.

IV. CONCLUSIONS

Sooner or later, the destiny of IoT becomes a really worth but big quantities of facts elevated its complexity in detection, communications, controller, and in producing cognizance however its boom can be elevated each day. Although destiny of IoT will be predictable to be integrated, all-in-one, and ubiquitous. carrier organization required to be enclosed in a hard and fast of requirements. So, As an smart device, progresses of IoT may be decided with the cooperation of interoperability, consciousness, professional, teamwork, energy, sustainability, privacy, believe, confidentiality, and safety. IoT have become an anticipated fashion of improvement of statistics industry. This will final results in pleasant of lifestyles. This paper surveyed a number of the maximum important issues and challenges of IoT in Indian attitude like what's being done and what are the issues that require similarly improvement.
Some feasible enhancements consist of including a facility to handle unified, seamless, and universal internet connectivity, standardization, with interoperability. Electricity sustainability, privateness, and protection are also essential factor on which research can go on. In the coming years, improving these challenges can be a effective and formidable step for networking and communities in industrial, industrial and academic region.

For the effective development technology of "Internet of things" is important to review the subsequent queries.

1) Regularization of such technology, in terms of ensuring security, providing specific specifications, including, for a distinctive course results, as a sample, "smart home" and therefore the like.
2) The majority of this state of technology of "Internet of things" to make sure the security involves the utilization of "forced" means that. It should be observed that the utilization of "imposed" solutions will typically be dearer than the system itself.

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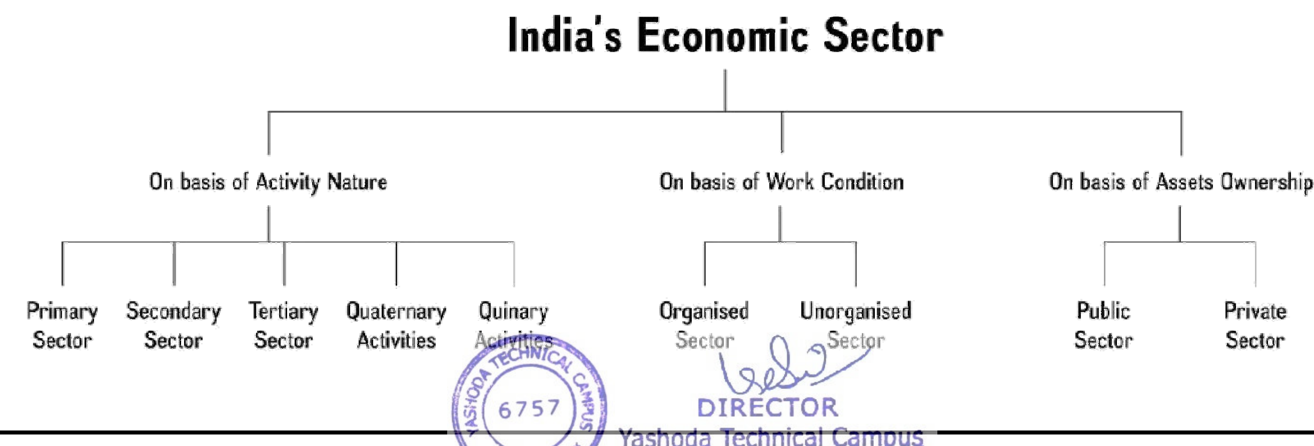
The Impact of Stock Market on Indian Economy

Omkar Namdev Devade[1], Vikas Chandrakant Gavali[2], Asst. Dr. Prof. S.P. Jadhav[3], Asst. Prof. S. V. Thorat[4]
[1, 2]MCA, YTC Satara
[3, 4]Dept. of MCA, Yashoda Technical Campus Satara, Maharashtra, India

Abstract: The Third Largest within the world of Indian Economy in terms of buying power. it's going to touch new heights in coming back years. The Global investment Bank , by once North American country and China 2035 India would third largest economy of the globe. it'll grow to hour of size of the North American country economy. This booming economy of nowadays must pass through several phases before it will bring home the bacon the current milestone of Sept. 11 gross domestic product. Movements within the stock exchange will have a profound economic impact on the economy and individual shoppers.
A collapse in share costs has the potential to cause widespread economic disruption. This paper deal s with stock market play very important role growth of Indian Economy and additionally the Impact stock exchange on Indian Economy by approach of Conceptual Methodology exploitation to the Journals of Indian stock exchange.

I. INTRODUCTION

A stock exchange, equity market or share market is that the aggregation of consumers and sellers (a loose network of economic transactions, not a physical facility or separate entity) of stocks (also known as shares), that represent possession claims on businesses; these may embrace securities listed on a public securities market, further as stock that's solely listed in camera.
Examples of the latter include shares of non-public firms that area unit oversubscribed to investors through equity crowd funding platforms. Stock exchanges list shares of common equity further as different security varieties, e.g. company bonds and convertible bonds. Stock market as argued by several economists is believed to exert Associate in Nursing impact on the economic growth of a nation for it provides a platform wherever capital can be raised for the institution of latest comes by firms or growth of their operations. As Osho (2014) noted, stock market plays a serious role as Associate in Nursing economic establishment which boosts potency in capital formation and allocation, it allows corporations and the government to raise long run capital that allows them to finance new comes or expand its activities. In support of the preceding argument, Jecheche (2011) sees the stock market to give the avenue for growing firms to raise capital at lower price and additionally, firms in countries with developed stock markets area unit less dependent on bank funding, which can scale back the danger of squeeze. Although stock exchange is seen as a stream for capital formation, its impact on economic process might not essentially be vital. Mark (2000) quoted economic expert as oral communication oral communication stock exchange isn't merely Associate in Nursing economical thanks to raise capital and advance living standards, however may be connected to a casino game or game of chance‖. economic expert arguments stem Stocks are classified in numerous ways that. One approach is by the country wherever the company is domiciled. For example, Nestlé and Novartis are domiciled in European country, therefore they might be thought about as half of land stock market, though their stock might also be listed on exchanges in different countries, for example, as yank repository receipts (ADRs) on US stock markets



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II. OBJECTIVE OF STUDY

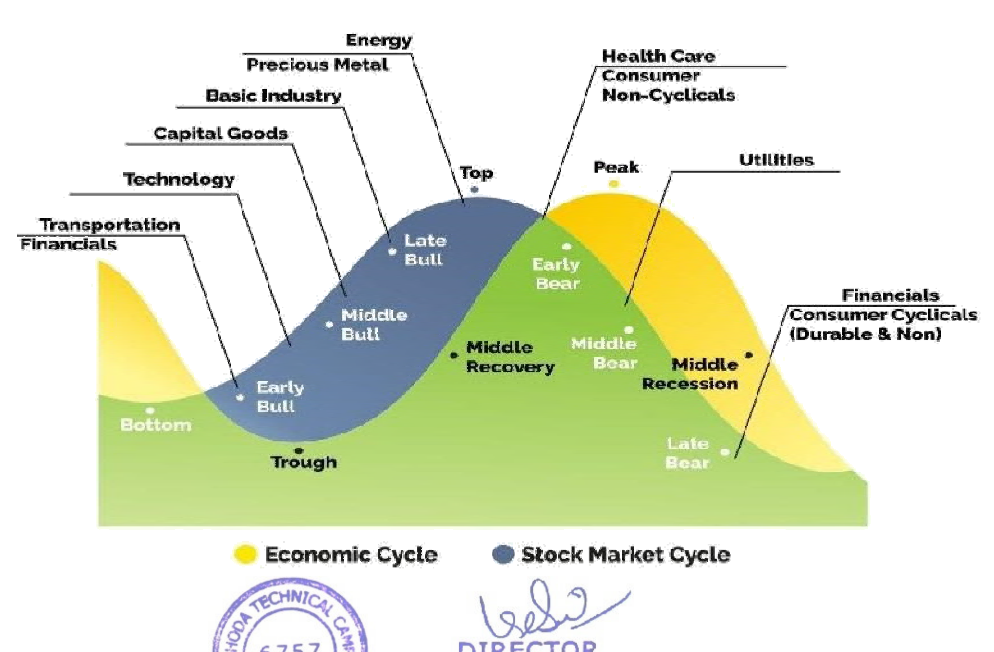
To know the impact of Stock Market in Indian Economy

III. METHODOLOGY

In this paper using secondary source of data like Journals, Magazines, Books, websites of Impact of Stock Market on Indian Economy. It is Conceptual Methodology.

IV. ROLE OF STOCK MARKETS IN THE ECONOMIC GROWTH OF INDIA

The role of stock markets as a supply of economic process has been wide debated. it's well recognized that stock markets influence economic activity through the creation of liquidity. Liquid money market was a very important enabling issue behind most of the first innovations that defined the first phases of the commercial Revolution. Recent advances during this space reveal that stock markets stay an important passage for enhancing development. many beneficial investments necessitate a semipermanent commitment of capital, however investors may well be reluctant to relinquish management of their savings for long periods. Liquid equity markets build investments less risky and additional enticing. At the same time, firms relish permanent access to capital raised through equity problems. By facilitating longer-term and additional profitable investments, liquid markets improve the allocation of capital and enhance the prospects for semipermanent economic process. what is more, by creating investments comparatively less risky, securities market liquidity may result in additional savings and investments. most major markets, ejection India's, closed 2018, having lost ground compared with a year past - wiping off billions in companies' capitalization. Unsurprisingly, the most important loser among Asian stock markets is China‘s, that was down twenty five.5 per cent for the year. this is often followed by Japan at fourteen.9 per cent and therefore the Philippines at fourteen.4 per cent. when a frantic 1st week of market activity in 2019, India‘s securities market that gained nearly seven per cent in 2018, is already down one per cent this year. Signs around the world area unit not promising. The US-China trade war is making uncertainty in international markets, combined by the anticipated speed of most economies round the world and adding fears that the USA Federal Reserve can still raise interest rates on issues of inflation. the worldwide economic retardation can not be higher illustrated than the darling of client technology, Apple, supplying a warning regarding slower sale of its phones than anticipated. This caused its shares to plunge ten per cent on weekday, wiping nearly $ seventy four billion off the market price of the once Most worthy company within the world. it's since recovered regarding four per cent on the ultimate commerce day of the week



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V. ECONOMIC EFFECTS OF THE STOCK MARKET

A. *Wealth Effect*

The first impact is that individuals with shares can see a fall in their wealth. If the autumn is critical it'll have an effect on their money outlook. If they're losing cash on shares they'll be additional hesitant to pay money; this may contribute to a fall in client disbursal. However, the effect shouldn't tend an excessive amount of importance. usually folks that obtain shares area unit ready to lose money; their disbursal patterns area unit usually freelance of share costs, particularly for brief term losses. The wealth impact is additional distinguished within the housing market. In Dec 2014, the worth of the united kingdom securities market was USA $6.06 trillion thus it's a giant impact on wealth.

B. *Effect on Pensions*

Anybody with a non-public pension or investment firm are plagued by the securities market, a minimum of indirectly. Pension funds invest a significant a part of their funds on the securities market. Therefore, if there's a significant fall in share costs, it reduces the worth of pension funds. this suggests that future pension payouts are lower. If share costs fall an excessive amount of, pension funds will struggle to satisfy their guarantees. The vital factor is that the long term movements in the share costs. If share costs fall for a protracted time then it'll positively have an effect on pension funds and future payouts.

C. *Confidence*

Often share worth movements area unit reflections of what's happening within the economy. E.g. a worry of a recession and international retardation might cause share costs to fall. The securities market itself will have an effect on client confidence. dangerous headlines of falling share costs area unit another issue that discourage folks from disbursal. On its own it's going to not have abundant impact, however combined with falling house costs, share costs will be a discouraging issue. However, there area unit times once the securities market will seem out of step with the remainder of the economy. within the depth of a recession, share costs could rise as investors foresee to a recovery 2 years within the future.

D. *Investment*

Falling share costs will hamper corporations ability to lift finance on the securities market. corporations World Health Organization area unit increasing and need to borrow usually do thus by supplying additional shares – it provides a coffee price means of borrowing more cash. However, with falling share costs it becomes rather more troublesome.

E. *Bond Market*

A fall within the securities market makes alternative investments additional enticing. folks could move out of shares and into government bonds or gold. These investments provide a stronger come back in times of uncertainty. although typically the securities market may well be falling over issues in bond markets (e.g. monetary unit business enterprise crisis) Stock markets area unit one in all the factors that have an effect on the economy, however there area unit others further. Interest rates have an effect on the economy as a result of rising rates mean higher borrowing prices. Consumer disbursal and business investment slows down, that reduces economic process. Falling interest rates will stimulate economic process. economic policy choices can also have an effect on the economy. For example, massive budget deficits will scale back government investments and purchases, that will slow down the economy. Currency fluctuations will come near the worth of exports, which may damage export-driven economies. Stock markets area unit one in all the factors that have an effect on the economy, however there area unit others further. Interest rates have an effect on the economy as a result of rising rates mean higher borrowing prices. client disbursal and business investment slows down, that reduces economic process. Falling interest rates will stimulate economic process. economic policy choices can also have an effect on the economy.

VI. CONCLUSIONS

Stock markets square measure one in every of the factors that have an effect on the economy, however there square measure others yet. Interest rates have an effect on the economy as a result of rising rates mean higher borrowing prices. client defrayment and business investment slows down, that reduces economic process. Falling interest rates will stimulate economic process. economic policy choices can also have an effect on the economy. For example, massive budget deficits will scale back government investments and purchases, that will slow down the economy.

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Web 3.0 – Future Of The Internet.

Sneha Sanjay Jadhav[1], Dattatray Apparao Gund[2], Asst.prof. S. V. Thorat[3]

[1st]*Sneha Sanjay Jadhav,MCA YTC, Satara*
[2nd]*Dattatray Apparao Gund, MCA YTC Satara*
[3rd] *Prof.S.V.Thorat Dept. of MCA Yashoda Technical Campus,Satara-415003*

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Abstract— **In an ongoing organized world, the Web has emerged as the most practical method of communication. During the early improvements of the Internet, there was a bit of thought that one day the progress of this Internet web would be a huge blow. In such a short time frame, Web 2.0 and now Web 3.0 have reached exceptional heights in the Internet industry. The split from Web 1.0 to Web 2.0 was advertised in virtually 10 years. However, shortly after Web 2.0, another Web 3.0 advance has increased interest and many inquiries from engineers, customers, and controllers. What is really needed at this stage, what are the driving variables, how unique are they in terms of Web 2.0 and the Semantic Web**

Keyword – Semantic web, web1.0, web 2.0, block chain, Decentralized

I. INTRODUCTION

Web 3.0 is a new era of the World Wide Web, where Web 2.0 innovations are closely linked to the Semantic Web, allowing both humans and machines to access and use data stored on the Web. With Web 3.0, machines actually have to perform tasks that require human insight, significantly reducing time and effort on the Internet. Web 3.0, aimed at making the Internet a better and smarter organization, is the predecessor of the complete Semantic Web and replaces Web 2.0. After quite a long time working on a centralized framework, the Internet will leap forward with the help of blockchain and its decentralized center. Migrating to Web 3.0 addresses the next stage of the Web, with freedom of information, practices, and common activity paths as standard. Web 3.0 innovation is returning privileges to customers by focusing on collaborative collaboration and discouraging collaboration with a unified organization. However, this change is difficult to understand, and of course many high-tech business visionaries and regular web consumers still don't know exactly what Web3 means. Web 2.0 provides important authority for collaborative use of the Internet, allowing individuals to connect to information and contribute to their perspective through wikis, web journaling, personal communication environments, and more. Models: Wikipedia, Blogger, Digg, Technorati, Stumble upon, MySpace, Facebook, Flickr and more. The idea behind the use of the Semantic Web is to capture and decode specific situations and ideas of information. Then, when the customer searches for an answer, Web3.0 provides the end customer with the most reliable and meaningful results. Therefore, this third era of the Internet is the era of evaluating customized connections to machines and websites, just as we are talking to other

II. What is Web 3.0

In the semantic web, the data is analyzed and interpreted in terms of context, concept, and relatability. Because of this web 3.0 applications can provide the most accurate and relevant results to the end-users.

Data are connected in a decentralized way-usually in a blockchain. This is a major leap forward from the current web 2.0's centralized architecture.

Web 3.0 is going to be considerably more secure, scalable, and offer better privacy for users. Most big tech companies make insane profits by exploiting user data as users have little to no control over it.

Web 3.0 will make it possible for users to have greater control and if they wish to share the data then to be compensated for it. As a result, users will retain privacy and ownership of their data while making it available for companies to target them.

Web 3.0 will also allow sites and applications to use data more effectively and tailor information to individual users.

III. KEY FEATURES OF WEB 3.0

The main aspect of web 3.0 is:

Open – "Open" in the sense that it was built using open source software, developed by an open and available community of developers, and realized for the public.

Untrusted – The decentralised network offers freedom to users to act publically associated in camera whereas not associate negotiator exposing them to risks, thus "trustless" info

No Permission – Anyone, including users and contributors, can contribute without the permission of the state body.

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Popularization – Web 3.0 makes the web offered anytime, anywhere. At some purpose, devices connected to the web are not any longer restricted to computers and smartphones, as in internet a pair of.0. With the IOT this technology allows the event of assorted new kinds of good devices. Web 3.0 could be a new generation of the globe Wide internet wherever internet a pair of 2.0 technology connects to the 3.0 sanctionative each humans and machines to access and use data hold on the net.

Web 3.0 allows machines to perform tasks that need human intelligence, considerably reducing time and energy on the net. With the goal of creating the web a stronger and smarter network, Web 3.0 is the forerunner of the whole linguistics internet and therefore the successor to internet a pair of 2.0.

Semantic Web: Semantics is the study of relationships between words, patterns, and data. In a semantic web, machines can analyze and establish relationships, just like humans do, between information on it.

A semantic web would consider both of the following two sentences same:

I love Programming

I <3 Programming

Semantics would help web apps to decode meaning, emotions, and hidden patterns to deliver a better online experience.

AI Driven: Web 3.0heavily relies on AI techs like Big Data, Data Analytics, Machine Learning, and Deep Learning. Apps and sites are getting smarter to understand the mood, emotions, and expressions of their users. Some websites are so advanced that they can even understand sarcasm!

In Web 2.0, this was mainly a human-driven rules-based process susceptible to corruption, bias, and oversight. The process was also too slow to meet the increasing demands of millions of users.

Ubiquitous: Ubiquity refers leads to accessibility, openness, transparency, and innovation. Not only the people with resources, but those with limited resources too can make use of Web 3.0 services.

As most of the processing is done at the backend, users can use even simple and cheaper devices to connect and transact.

Spatial and 3D Graphics: Web 3.0 is going to be more spatial thanks to 3D graphics and AR/VR innovations. The lines between the real world and the cyber world have started to blur as multiple new 3D virtual worlds evolve. Immersive technologies like Augmented Reality (AR) and Virtual Reality (VR) are used in gaming, medicine, engineering, tourism, education, and many other areas.

IV. HOW DOES WEB 3.0 WORK ?

The idea behind web 3 is to make glancing through the Internet lots quicker, less complicated, and extra talented to cope with even complicated hunt sentences inside the blink of an eye.

In an internet 2.0 utility, a purchaser desires to cooperate with its frontend, which imparts to its backend, which in addition speaks with its facts set. The entire code is facilitated on included servers that are shipped off customers via an Internet program.

Web 3 has neither focused facts bases that keep the utility country nor an included net server in which the backend intent dwells. All matters being equal, there's a blockchain to manufacture packages on a decentralized country system and stored up via way of means of unknown hubs on the net.

V. EVOLUTION OF WEB

(1.0 TO 2.0 TO 3.0)

1. Web 1.0 :_

In the 1960s, Web 1.0 was a static format with just text browsers like ELISA, followed by HTML, which improved the visual attractiveness of the pages, and the first visual browsers like Netscape and Internet Explorer. Web 1.0 is the first stage of the World Wide Web's evolution. There were formerly only a few content creators. On the other hand, most users on Web 1.0 were content consumers.

2. Web 2.0:-

Tom OReilly invented the phrase Web 2.0 in 2004 to describe the second generation of website models. Websites that emphasise user-generated content, ease of use, and interoperability for end users are referred to as Web 2.0.

3. Web 3.0:-

With technologies like AJAX, Web 3.0 originally debuted in 2006 in an article by Web 2.0 critic Jeffrey Zeldman. Web 3.0 is a concept that refers to a number of advancements in web usage and cross-path interaction. In this case, the data is shared rather than owned, and services display diverse views of online data.

VI. COMPONENTS

The web 3 contains the principles and apparatuses of XML, XML Schema, RDF, RDF Schema and OWL that are coordinated in the Semantic Web Stack. The Web Ontology

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Language Overview depicts the capacity and relationship of every one of these parts of the web 3.0:

1. XML gives an essential grammar to content design inside archives, yet connects no semantics with the importance of the substance held inside. XML isn't at present an important part of Semantic Web innovations by and large, as elective linguistic uses exists, like Turtle. Turtle is a true norm, however has not experienced a proper normalization process.

2. RDF is a basic language for communicating information models, which allude to objects ("assets") and their connections. A RDF-based model can be addressed in XML language structure.

3. RDF Schema broadens RDF and is a jargon for depicting properties and classes of RDF-based assets, with semantics for summed up pecking orders of such properties and classes.

4. OWL adds more jargon for depicting properties and classes: among others, relations between classes (for example disjointness), cardinality, correspondence, more extravagant composing of properties, qualities of properties (for example evenness), and listed classes.

VII. BENEFITS OF WEB 3.0

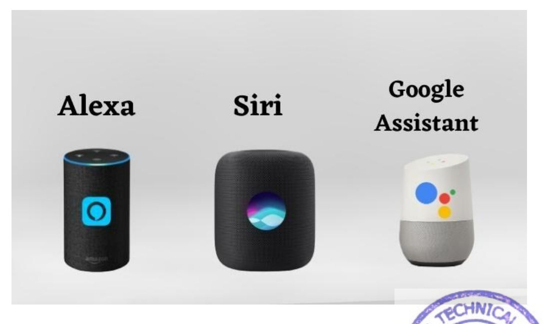
Web 3.0 it is decentralized is a word used to describe a system where no single person or group has control it's the opposite of centralized where one entity controls everything decentralization has been around for quite some time and now more than ever before we're realizing the true potential it's faster web3 technology is a new and improved version of the current technology.

VIII.BEST WEB 3.0 APPLICATIONS

Web 3.0 is already being deployed to a variety of uses and apps in multiple industries. The cutting-edge technologies increase productivity and enhance customer satisfaction.

Let us examine some of the best applications:

1)Siri, Alexa, and Google Assistant:



The voice assistants from the top three tech companies in the world – Siri from Apple, Alexa from Amazon, and Google Assistant from Google – use the semantic web. This software leverage voice recognition and natural language processing to help users do things they could not do earlier. Today these assistants can offer answers to a variety of questions from their users.

2)Facebook Meta:

If it were a nation, it would be the most populous one on the earth. The leading social network platforms Facebook and Instagram from the Meta are increasing in their reach exponentially and impact users' lives daily. Users find and create new communities and bonds with the help of Web 3.0 technologies. Apps build around the Facebook universe further increase customer interaction and engagement.

3)Flicker:

The photography and photo-sharing website Flickr allows users to search, create, upload, and share their pictures with people they care about. With over 17 million active visitors per month, Flickr has one of the largest public databases with thousands of categories and billions of photos in them.

IX. FUTURE OF THE INTERNET:

Web 3.0 creates an ecosystem for users, by users, and of users. The end-users would be in complete control of their data on the internet and will drive the business of the future.

The creator-driven economy where creativity, innovation, and uniqueness rules would be supported by technologies of

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Web 3.0. according to use, the future of the internet is bright because of the following reasons:

- More trustworthy because of decentralized public records
- No more dependent on centralized authorities and data repositories
- Personalized interactions with users
- Faster and superior search results driven by AI
- No more dependency on mediators
- More peer-to-peer communication and connectivity

X. CONCLUSION

Web 3.0 is about the web's backend, about developing tremendous machine-to-machine communication. When the Web 3.0 ui gains traction, it will fundamentally change how we use the Internet. Humans will no longer be required to conduct tough jobs such as conducting Internet research and retrieving precise information.

All of these duties will be performed more easily by machines. All we have to do now is examine the data, update it as needed, and build any new object we want.

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Robotics: Social Robot

Vijay Lokhande[1], Suraj Shinde[2], Vanmala Kadam[3], Snehal Jadhav[4]
[1, 2]Students, [3, 4]Assistant Professor, MCA Department, MCA Department, Yashoda Technical Campus , Satara

***Abstract:** Social robots are is also called to 'family robot' .In a social Robots are the study which provides to the communication about them selves . it is ourselves with people, and with the environment, within the joined to its role. This paper discusses the concept of a social robot. To the Current. Social robots also used in house and health care.*

Than the world's first social robot designed by MIT robotics professor Cynthia Breazeal, the Jibo robot is often described as "the world's first family robot". Social Robots are helpful in health care and domestic areas, and in education and language learning's, arts and entertainments. In this aim is to create guideline, an intended design for future developments of social robot. In this research paper the researcher has discussed about the introduction part of the social robots, applications of social robots a research has also focus on Benefits limitation and the future working of the social robots. In overall conceptual discussion of social robots the research has also focuses on future development.

***Keywords:** Social Robot , Robotics , Human robot Interaction , Learning , Healthcare, Education.*

I. INTRODUCTION

A Social robots is a artificial intelligence system that is designed to communicate with humans and other robots. It is the robots which having an it's own laws. And which contains human interaction. Social robotics is a recently branch of robotics.

Social robots can interacts with humans it's rules and regulations which is joined to it's role. Some artificial social agents are designed with a screen to represent the head or 'face' in a constant change which is communicate with users

A. What are Social robot?

Unlike the robots that have became a familiar view in factories and warehouse, which have only limited contact with humans. Social robots are designed to communicate with us. Nowadays "People don't want to work with robots, because they're going to lose their jobs."

B. What is the use of Social Robots in Today's World?

To the some of the way to social robot are used to today include was their in taken to tutoring - provide learners with a fun is to responsive in a practice and master was new learning skills and for health care target in .

C. Use case for a Social Robots

In early in generations of the social robot in were designed for not dependent and similar tasks, such as expanding the ocean floor, providing the produce the process or helping to fulfill warehouse orders. Some of the another ways that social robots are used today include:

1) *Tutoring:* This provides learners with a fun, responsive way to practice and master new learning skills.
2) *Companionship:* In the provide to emotional support young , the elderly or disabled.
3) *Customer Engagement:* Social robots provides an possible customers with information about products and services, store hours and pricing.

II. LITERATURE REVIEW

1) Joseph E Michaelis and Bilge Mutlu are the author of "Supporting Interest in Science Learning with a Social Robot"(June 2019) in that experts are studied on basics robots .In Interaction Design and Children (IDC '19) June 12–15, 2019, Boise, ID, USA , New York, NY, USA. In this research paper education researchers and learning scientists have emphasized that impactful student learning requires than the , it was knowledge the goes simple acquisition of facts and procedure.
2) Tony Beldame and James Kennedy are the author of "Social robots for education: A review"research paper.The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim in the original Government Works. They have been shown to be effective at increasing the cognitive and affective outcomes and have achieved by outcomes equal to those of human tutoring on limited tasks.

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III. TOP 8 COMPANIES WHICH PRODUCES THE SOCIAL ROBOTS ARE AS FOLLOWS

1) Scandit
2) Knightscope
3) Furhat Robotics
4) Intuition robots
5) Intelligent Automation,Inc
6) Google
7) TNO
8) Reach Robotics

IV. APPLICATIONS IN SOCIAL ROBOT AN DIFFERENT SECTORS:-

A. *Social Robots in Education Sector*

The social robots in educational sector is used for teaching purposes and employing them in robotic engineering and programming courses.

Children love interacting with a robot that talks back to them, and people are seeing excellent results in happiness and communication skills for autistic children and students with learning difficulties.

B. *Social Robots in Home and Food Services Sector*

The Social robots in home sector is used in kitchen. It is used to provide receipes and more food information. It is also to help to make special receipes and to serve the food to your family members. In this sector mostly social robots are used for purposes

C. *Social Robots in Healthcare Sector*

The Social robots in health sector are used everywhere from science fiction to your local hospital. It is used in healthcare for medicinal use for help by relaxing medical people's from daily tasks , that take their time away from more pressing responsibilities, and by making medical procedur .

V. SOCIAL ROBOTS IN ARTS AND ENTERTAINMENTS SECTOR

The Social Robots are also plays an main role in arts and entertainments sector. Social robots includes different arts and also a nice entertainer for kids and elder people's.Different entertainment's like Social robots can play, dance and talk.

VI. ADVANTAGES OF SOCIAL ROBOTS

1) Social robots can be also used in the classroom settings and in the tutor and learner motive.
2) Social robots are less afraid than humans to children with autism.
3) Social robots are faster and more accurate and less noisy.
4) Those who don't have time Ex: Mothers doing chores.Then they can focus more on children and also they are also helpful in serving the food.
5) Social robots in the shops are interacting with customers and imparting knowledge about a product range, presenting a particular item or item in a certain size

VII. DISADVANTAGES OF SOCIAL ROBOTS

1) Social robots are more costly and also they takes an more time for procedur and they are placed under striker guidelines and which is not affordable to common person.
2) More training is needed for the social robots to operate the whole system.
3) Social robots need to supply of power, they need maintainence to keep them running.

This procedure is to restore the lost code or data may be time-wasting and costly.

VIII. IMPORTANCE AND NEEDS OF SOCIAL ROBOTS

Most social robots today are used to do repetitive actions or many household or workplaces jobs which considered as relax for humans. A social robot is ideal is for the various sectors like education sectors, home sectors, healthcare sectors and for retail and shops sectors. Social robots are to make in our work easy and simple to importance. Therefore, the need of social robot is most important in today's world

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IX. IMPACTS OF SOCIAL ROBOTS IN EVERYDAY LIFE

A. Positive Impacts

The most important positive impact of social robots is that social robots reduces the man power. By helping the humans in every house hold works.And the safety is the most important advantage of using robotics.

Social robots contains perfection and robots will always deliver quality.Social robots makes employees happier and also maintains a job and productivity.

B. Negative Impacts

They cannot handle unexpected situations.

Social robots contains AI but they are not as intelligent as humans. They cannot think for own and also they can never develop their duties outside the pre-defined programming because they simply cannot think for themselves. And also social robots have no feel of emotions.

X. FUTURE CHALLENGES

In spite of the effective children engagement with social robots in special education, for the health of the children's with autism and old aged person's and for the home motive and working in the retail and shops.

There are following some specific challenges in improving the interaction experience and in increasing the above workouts.

1) When finding in condition for a seeking the attention with ASD (autism spectrum disorder.
2) Motivate the verbal communication during human-robot interaction.
3) Also Develops multi-robot and multi-child interaction condition in education and learning purposes.
4) It is the mostly important future challenge on to social robot is replaced by the human for the kitchen purposes to done the work in easy way.
5) In coming days social robots are most important in the businesses and various retails and shops purposes.
6) It is worth to note the above contained challenges deal with the improvement of the overall child-robot interaction ,human robot interaction specially for education ,healthcare, home, retails and shops purposes as a communication.

XI. CONCLUSION

It is concluded from the above discussion that the social robots are mostly used and important in the human-robot interaction. An the social media robots are designed in a reduced to the tasks of the human's in the different sectors.

There is much needs of the social robots in our daily life and in our daily workout of humans. The social robots it take can interacts in with humans by following social behaviors and it's rules and regulation it was to which is attached to it's role. Social robots are used in various fields for education or education,

housing and food services, health care, arts and entertainment, retail outlets and shops, and for information and communication purposes. Therefore, by this developed technologies the for development of nations and to reduces the work of humans. So, overall in this research we can include the abstract, introductory part, applications, use cases, importance and needs, positive and negative impacts, future challenges, etc.

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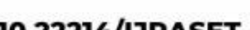

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Virtual Smart Phones

Rutuja Suryakant Deshmukh[1], Vijaya Mahendra Mohite[2], Prof.Dr.S.P.Jadhav[3], Prof.S.S.Jadhav[4]

[1st]Rutuja Suryakant Deshmukh, MCA YTC, Satara
[2nd] Vijaya Mahendra Mohite, MCA YTC Satara
[3rd] *Prof.Dr.S.P.Jadhav,[4th] Prof. S.S. Jadhav Dept. of MCA Yashoda Technical Campus,Satara-415003*

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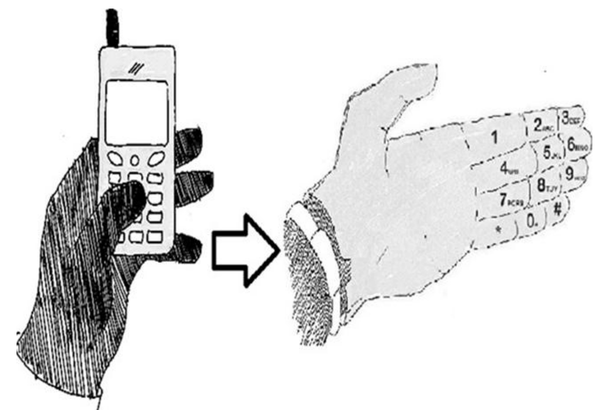
***Abstract*:-** For every departure metal money communication may be a manner by that they share/pass their thoughts/fillings to 1 another. we have a tendency to homosepians chiefly use verbal communication to speak with one another. during this Paper we have a tendency to introduce VSP, a Virtual good Phone that is largely a step to attach each the Physical and virtual world, by employing a little projector, Camera, Speaker, microphone & Cloud Computing Technology over the net within the kind of wearable device. In VSP all the specified element area unit fancied within the wearable device by that use communicate with the assistance of natural hand gesture, Hand movement and net. In VSP user communicate with one another by Virtual mobile with the assistance of bit gesture electromagnetic radiation and cloud computing technology.

VSP can finish the physical dependency of mobile. VSP offer novel interaction methodology to seamlessly communicate with one another in an exceedingly fun and intuitive manner. The user will bit their Palm to form decision and might even be used for looking at movies or pictures on their Palm/wrist. bit gesture is employed for creating and Terminating the decision. VSP uses touch-based interactions as instruction for establishing communication between the various users.

1. INTRODUCTION

The recent advent of novel sensing and show technologies has inspired the event of a spread of multi-touch and gesture primarily based interactive systems. In these systems user could move directly with info victimization bit add natural hand gestures. these days there area unit voluminous approach by that we will hook up with digital world within the controlled surroundings victimization muti-touch and gesture primarily based interaction. sadly, most gestural and multi-touch primarily based interactive systems don't seem to be mobile and little mobile devices fail to supply the intuitive expertise of full-sized gestural systems.

Moreover, info still resides on screens or dedicated projection surfaces. there's no link between our interaction with these digital devices and interaction with the physical world around US. during this paper, we have a tendency to gift VSP-Virtual sensible Phone, a multi-touch and gesture primarily based interaction system. that replace the physical transportable device to the virtual multi-touch & natural gesture primarily based interaction on the user palm by that user communicate with alternative digital devices over the network. VSP primarily turns the human hand as a transportable by that is ready to user hook up with the digital world additionally as alternative peoples like their friends and relatives.



VSP is largely a computer-vision primarily based wearable and gestural info interface that augments the physical world around US with digital info and proposes natural hand gestures because the mechanism to move therewith info.

2. RELATED WORK

Recently, there are an excellent form of multi-touch interaction and mobile device merchandise or analysis prototypes that have created it doable to directly manipulate computer programme parts victimisation bit and natural hand gestures. Most of those systems rely upon the physical bit-based interaction between the user's fingers and physical screen and therefore don't acknowledge and incorporate touch freelance freehanded gestures. VSP Virtual sensible Phone Technology takes a unique approach to computing and tries to form the digital facet of our lives a lot of intuitive, interactive and, above all, a lot of natural. It's plenty of advanced technology squeezed into a straightforward moveable device. once we herald property, we are able to get instant, relevant visual info projected on any object we tend to develop or act with the technology is especially supported hand increased reality, gesture recognition, laptop vision based mostly formula etc.

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Augmented Reality

Augmented reality (AR) may be a term for a live direct or indirect read of a physical globe setting whose components square measure increased by virtual computer-generated imagination. it's associated with a a lot of general idea referred to as mediate reality during which a read of reality is changed (possibly even diminished instead of augmented) by a laptop. The augmentation is conventionally in time period and in linguistics context with environmental components.

Virtual good Phone uses increased Reality idea to position digital info on the physical world. With the assistance of advanced AR technology (e.g. adding laptop vision and object recognition) the data concerning the encompassing globe of the user becomes interactive and digitally usable. Artificial info concerning the setting and therefore the objects in it are often keep associate degreed retrieved as an info layer on high of the important view. the most hardware elements for increased reality are: show, tracking, input devices, and laptop. Combination of powerful hardware, camera, accelerometers, GPS and solid state compass square measure typically gift in fashionable Smartphone, that create them prospective platforms

Gesture Recognition

Gesture recognition may be a topic in engineering science and language technology with the goal of decoding human gestures via mathematical algorithms. Gestures will originate from any bodily motion or state however normally originate from the face or hand. Current focuses within the field embrace feeling recognition from the face and hand gesture recognition. several approaches are created mistreatment cameras and pc vision algorithms to interpret signing. Gesture recognition will be seen as how for computers to start to know soma language, so building a richer bridge between machines and humans than primitive text user interfaces or perhaps GUIs (graphical user interfaces), that still limit the bulk of input to keyboard and mouse. Gesture recognition allows humans to interface with the machine (HMI) and act naturally with none mechanical devices. Gestures will be wont to communicate with a pc thus we are going to be largely involved with empty handed semiotical gestures

Computer vision Based Algorithm

Computer vision is that the science and technology of machines that may see. As a study, laptop vision worries with the idea behind artificial systems that extract info from pictures. The image information will take several forms, like video sequences, views from multiple cameras, or multi-dimensional information from a medical scanner. The software system tracks the user's gestures mistreatment computer vision primarily based algorithms. the pc vision system for pursuit and recognizing the hand postures that management the menus relies on a mixture of multi-scale color feature detection, read primarily based stratified hand models and particle filtering. The hand postures or states ar depicted in terms of hierarchies of multi-scale color image options at totally different scales, with qualitative interrelations in terms of scale, position and orientation. In every image, detection of time period color options is performed.

The hand postures ar then at the same time detected and half-tracked mistreatment particle filtering, with associate degree extension of superimposed sampling stated as stratified superimposed sampling. to boost the performance of the system, a previous on skin colour is enclosed within the particle filtering. Figure 2: Gesture Recognized Mobile keyboard VSP conjointly|is additionally} associated with increased reality wherever digital info is superimposed on the user's read of a scene however it also take issue in many vital ways that. 1st VSP permits user to move with the projected info mistreatment hand gestures. Second the knowledge is projected onto the Hand/object and surfaces themselves, instead of onto glasses, spectacles or watch which ends in a {very} very totally different user expertise.

3. OBJECTIVE

VSP Invention is said to transfer of knowledge & establishing communication from one physical body to different physical body or from one physical body to digital devices or vice-versa with none platform dependency. VSP is essentially AN makes an attempt to create the communication between users and Digital devices additional tangible and interactive. the target of this invention is establishing the connection/communication between humans and conjointly with digital devices by barely gesture on the human Palm/Hand. VSP work on 2 kind of information transfer.

First, It establish auditory communication between the users with the assistance of GSM Technology with none physical cell phone.

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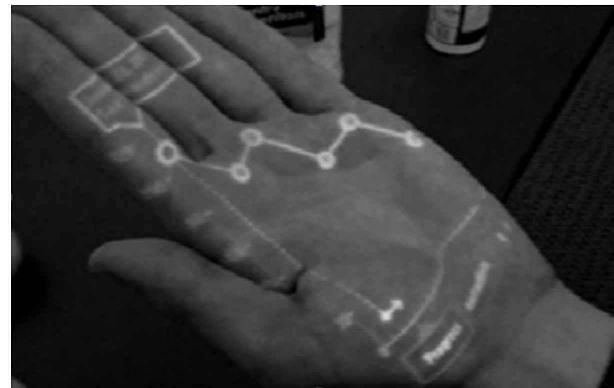
Second, For Transfer of knowledge between the humans and conjointly with digital devices. It create use of the net, computer network network or the other kind of information Servers through that device and humans area unit connected to and also the distinguish from one user to a different by the authentication ways like username/password, drawing a pattern on the virtual screen, face recognition, Palm recognition victimization palm lines or fingerprint detection will be used. In VSP auditory communication type one human to a different will be done either by victimization GSM or Internet/Intranet technology.



The Transferring of knowledge from one creature to a different or device victimization VSP. the primary and second digital devices is also gesture recognition VSP system connected to a network as well as a knowledge storage cloud and each uses VSP Technology.

4. WORKING

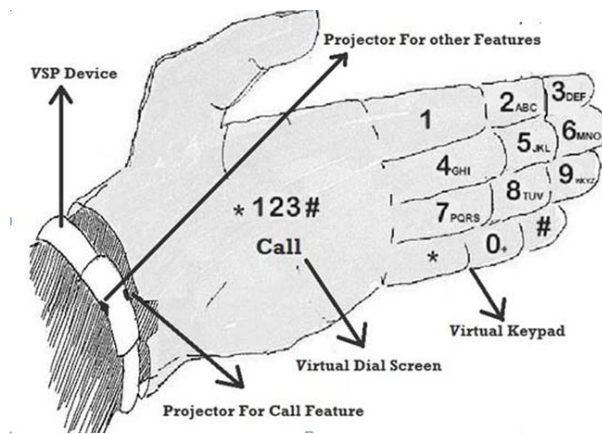
Working of VSP accommodates five Main steps i.e. sanctioning & evidence VSP, Make Call, Receive decision, Capture Image/Video, repetition knowledge & paste/Pass knowledge to different VSP & Digital Devices as follows.

A. sanctioning VSP

The VSP may be a wearable device and user has the key to change (ON)/Disable (OFF) the device through the ability Button. once user change the VSP Device, associate icon seems on the user palm or arm as per user as per designated by the user for showing the standing (if a user has signed in).If not user will bit this icon to login or modification users victimization totally different authentication strategies like: Enter user name and secret, Drawing a secret sign or pattern, Face recognition, image choice and Fingerprint detection and Palm line Detection once a user has signed in with success, VSP is currently prepared for creating and receive calls and different Operations

B. Make Call

After enabling VSP currently user is in a position to create decision and communicate with their relatives and alternative persons. to create decision, Dial mobile range mistreatment virtual key or mistreatment Voice Recognition system. For establishing decision between 2 users, VSP uses 2 technique that ar as follows.

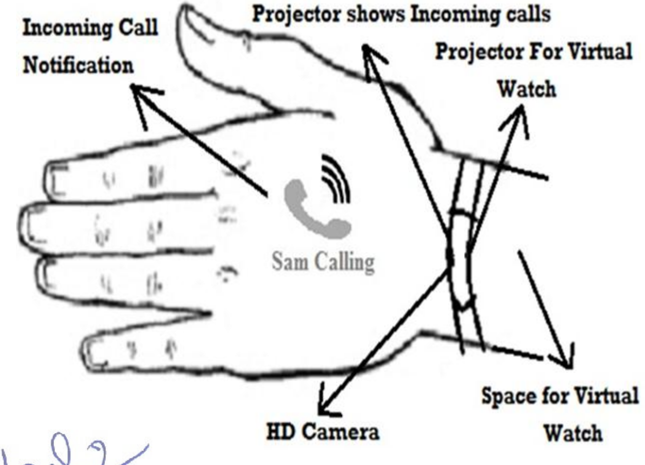


a. build decision mistreatment SIM:

VSP device encompasses a small SIM (Subscriber Identity Module) by that device established the decision mistreatment GSM/CDMA (Global System for Mobile Communications / Code Division Multiple Access) Technology.

b. build decision mistreatment VOIP:

VSP device encompasses a Wi-Fi (Wireless Fidelity) and Mobile information possibility that connect the device to the Intranet/Internet, by mistreatment this user is in a position to create calls mistreatment VOIP (Voice Over IP) Technology. By mistreatment VOIP user is in a position to create the decision to alternative VSP user additionally as all the others GSM and net VOIP change Digital Devices. once user isn't connected to internet/Intranet, decision is just created mistreatment SIM while not user's permission however once user hook up with net it raise user to pick the choice by that user needed to create decision as per user choice the decision is hook up with alternative person.



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C. Receive decision

When a VSP user referred to as by different VSP user or different digital device users by (Physical portable laptop computer, Desktop and personal organiser 'Personal knowledge assistant) the notification of incoming decision are going to be shown as per user designated Profile if user choose vibrate mode, the tiny vibrator motor indicate incoming decision by vibration & conjointly shows the identity of occupation user on back aspect of palm victimization high Density projector of VSP. If user choose Sound Mode, incoming decision notified by designated ring tone with user Name on the rear aspect of Palm. In Silent mode it solely indicate the name of caller within the back aspect of palm. For attending the incoming decision user simply bit, swipe the incoming decision icon or different bit gesture designated by user. to talk the caller user either use Bluetooth telephone receiver or wired telephone receiver that is connected to VSP device victimization three.0 connective. User is also ready to receive decision directly victimization VSP Device Speaker and Mice. For VOIP calls each user should be connected to the net victimization WI-FI or Mobile knowledge.

D. Capture Image/Video

VSP is additionally ready to capture prime quality Images/Video victimization their prime quality Camera by click capture image button or by victimization gesture (make a fame victimization our index figure and thumbs) for taking photos. once taking the image it shows the image on user hand victimization VSP System. For shoot video with constant gesture user simply needed to alter the camera mode photos to video. User conjointly center or zoom out whereas they capture Image/Video victimization their hand gesture.

E. Copy Data:

In VSP permit users to Transfer (Copy/Paste) knowledge from one shape to a different shape or device by employing a single bit gesture. For copy knowledge user must login initial in VSP device and connected to Internet/Intranet. For distinctive a duplicate event in VSP uses an extended press (Detect by perceiver Program) on copy ready knowledge item (keeping finger on a knowledge item quite one.5 sec. shown on user arm victimization VSP projector) indicates to repeat that knowledge item. Whenever user bit any copy ready knowledge barely perceiver program begin investigating the time and once time exceeds the edge (1.5 sec.) a message seems indicating that {the knowledge|the info|the information} item is being traced and gets traced to the user's distinctive area within the data cloud. The copy knowledge to the information cloud may also be done by other ways (instead of long-press for one.5 seconds). for instance, double faucet on knowledge item or draw a circle the information item to initiate copy. victimization this method user copy multiple file for passing/paste to the opposite device all the copy knowledge save within the cloud on temporary bases with distinctive id of every knowledge item.

5. TECHNOLOGIES USED

VSP is largely a wearable device that is combination of hardware still as software system. In hardware VSP incorporates Processor Unit, Ram & storage Memory, Power provide (battery), Sensors (Accelerometer, sixteen Proximity sensing element for distinguishing bit on Arm), light-emitting diode Indicator For Device Mode (ON/OFF), small Vibrator Motor, USB port (For charging or attaching different devices), four small Projectors (like Pico Projectors), one HD Camera for Capturing pictures and videos, Low energy needed WI-FI and Bluetooth devices, GPS system, four bit buttons (ON/OFF Button, Snap Button, sound Up button, sound down button) and Nano SIM card slot. In software system it use gesture recognition system, bit based mostly interaction system, increased Reality, laptop vision based mostly formula to meet all the objectives.

VSP uses the subsequent Technology for create decision, Receive decision, repetition knowledge & paste/Pass knowledge to different VSP & Digital Devices.

a. Voice Call:

In VSP voice decision done by exploitation either by exploitation SIM (GSM/CDMA) or although net exploitation VOIP Technology.

b. knowledge Transfer:

Data transfer from one body to a different body or device in exploitation VSP is completed by exploitation knowledge Cloud. For Accessing knowledge cloud user is also connected to net either by WI-FI or Mobile knowledge exploitation SIM.

6. CONCLUSION

VSP is essentially a computer-vision primarily based wearable and gestural interface that augments the physical world around United States with digital info and proposes natural hand gestures because the mechanism to move thereupon info. It connect Physical world to Virtual world. VSP provide intuitive thanks to communicate and knowledge Transfer between completely different|completely different} users similarly as different Digital Devices.

VSP invention fulfill our 2 future necessities. First, it's free morpheme physical dependencies of devices. Second, it connect our physical world to virtual world Some Application of VSP as Follows:

1. employed in Health watching System.

2. accustomed realize info of any Product/Item.

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3. accustomed Connect News and Weather Update.

4. accustomed connect completely different Devices just about.

5. employed in Education & coaching system.

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Overview of Social Media

Akanksha Potdar[1], Pragati Nagulkar[2], Prof. Snehal Jadhav[3]
[1, 2, 3]Department of MCA, Yashoda Technical Campus, Satara, Shivaji University, Kolhapur

Abstract: For young people, social media provides Friends we could not see in person were available online and allowed us important points of connection. On the other hand, fewer opportunities to interact in person with friends and family meant less real-world scrutiny on some of the negative effects of social media. Whether it's social media or in person, a good peer group makes a difference. a platform to help them discover who they are. For very shy or introverted youth, this can be a way to meet other people with similar interests. During the pandemic, social media made it possible for people to connect in ways when in-person socialization was not possible. Social support and socialization are important influences on coping and resilience.

I. INTRODUCTION

Social media is responsible for increasing mental health problems. This systematic study summarizes the effects of social network use on mental health. Social media activity like spending time together shown to have a positive effect on mental health domains. However, due to the cross-sectional design of the sample and methodological limitations, there are considerable differences. The composition of social media effects on mental health needs to be further analysed through qualitative research and vertical cohort studies. Man is a social animal who needs the co-operation of others to progress in life. Thus, being socially connected with other people can relieve stress, anxiety and sadness, but a lack of social connectedness can pose serious risks to mental health. Social media has recently become a part of people's daily activities. Many of them spend hours every day on Messenger, Instagram, Facebook and other popular social media. For teenage girls in particular, the more time they spend on social media is directly related to how much they absorb the idea that being thin is the norm, motivated to try to be thin and/or scrutinize their bodies excessive

A group of friends who connect over shared interests like art or music, and who are balanced in their outlook on eating and looks, is a positive. If you think social media is a negative experience, you may need a break. It's more difficult to permanently disconnect from social media—especially for young people. These platforms are powerful tools for connecting and staying up-to-date with friends and family Social events too. If you are not on social media then you are depending on your friends to contact you in person, which doesn't always happen.

The figure below shows news consumption by US adults on social media.

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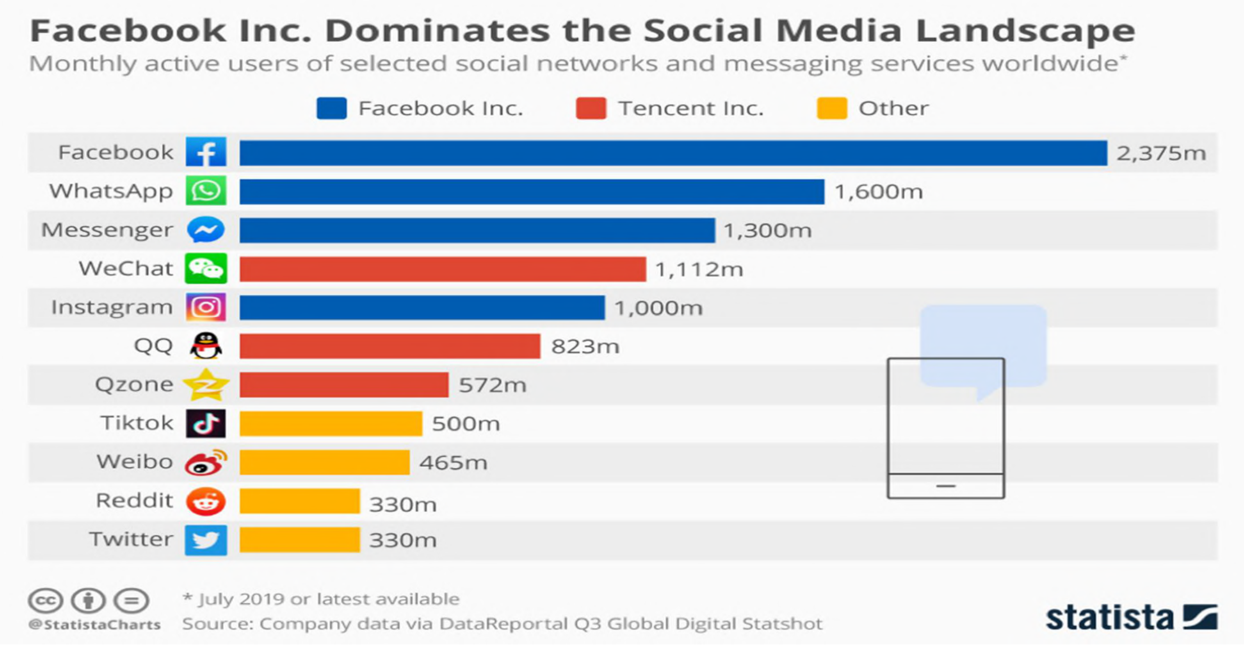
II. NEED FOR A SYSTEMATIC REVIEW

Systematic studies can identify, aggregate, and evaluate all accessible data to generate a warm and precise response to the research questions involved, both quantitatively and qualitatively [4]. Furthermore, there are many existing systematic studies related to mental health studies around the world. However, only a limited number of studies are integrated with social media and conducted in a social science context as the available literature is heavily focused on medical science. Because social media are a relatively new phenomenon, the relationship between their use and mental health.



III. CONCLUSION

Though it is a fictional statement, but still we should see social media as a boon for mankind. We need to understand that social media is for humans and not vice versa.

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Blue Brain Technology

Nikita Waydande[1], Priya Shinde[2], Vanmala Kadam[3]
[1, 2]Student MCA Department, [3]Assistant Prof. YTC Satara, Shivaji University, Kolhapur

Abstract: "Blue brain" is the name of the world's first virtual brain. It allows to transfer all the substances of human brain to virtual brain like PC. That means a machine can function as human brain. In other words, human is does not live for thousands of years but the information in his mind could be saved and used for several thousands of years. Today scientists are in research to create an artificial brain that can think, response, take decision, and keep anything in memory. The main aim is to upload human brain into machine. So that man can think, take decision without any effort. After the death of the person the virtual brain can store the knowledge, intelligence, personalities, feelings and memories of that person that can be used for the development of the human society.
Keywords: Blue Brain, Brain, Neurons, Sensory System

I. INTRODUCTION

This Human brain, the most valuable creation of God. The man is called intelligent because of the brain. Today we are developed because we can think, that other animals can not do .But we loss the knowledge of a brain when the body is destroyed after the death of man. That knowledge might have been used for the development of the human society. What happen if we create a brain and up load the contents of natural brain into it. This BLUE BRAIN project was founded in May 2005 by Henry Mark ram at the EPFL in Lausanne, Switzerland.

Blue Brain "The name of the World's first virtual brain. That means a machine that can function as human brain. Today scientists are in research to create an artificial brain that can think, response, take decision, and keep anything in memory. The main aim is to upload human brain into machine. So that man can think, take decision without any effort. After the death of the body, the virtual brain will act as the man .So, even after the death of a person we will not loose the knowledge, intelligence, personalities, feelings and memories of that man that can be used for the development of the human society. No one has ever understood the complexity of human brain. It is complex than any circuitry in the world. So, question may arise is it really possible to create a human brain? The answer is Yes. Because what ever man has created today always he has followed the nature. When man does not have a device called computer, it was a big question for all .But today it is possible due to the technology. Technology is growing faster than every thing.

II. OVERVIEW OF BLUE BRAIN

The IBM is now developing a virtual brain known as the Blue brain. It would be the world's first virtual brain. Within 30 years, we will be able to scan ourselves into the computers. We can say it as Virtual Brain i.e. an artificial brain, which is not actually a natural brain, but can act as a brain. It can think like brain, take decisions based on the past experience, and respond as a natural brain. It is possible by using a super computer, with a huge amount of storage capacity, processing power and an interface between the human brain and artificial one. Through this interface the data stored in the natural brain can be up loaded into the computer. So the brain and the knowledge, intelligence of anyone can be kept and used for ever, even after the death of the person.

III. WORKING OF HUMAN BRAIN

A. Sensory Input

When our eyes see something or our hands touch a warm surface, the sensory cells, also known as Neurons, send a message straight to your brain. This action of getting information from your surrounding environment is called sensory input because we are putting things in your brain by way of your senses.

B. Integration

Integration is best known as the interpretation of things we have felt, tasted, and touched with our sensory cells, also known as neurons, into responses that the body recognizes. This process is all accomplished in the brain where, many neurons work together to understand the environment.

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C. Motor Output

Once our brain has interpreted all that we have learned, either by touching, tasting, or using any other sense, then our brain sends a message through neurons to effecter cells, muscle or gland cells, which actually work to perform our requests and act upon our environment.

IV. NEED OF BLUE BRAIN

Now the world is very much developed because of the intelligence we have which is an inborn quality and cannot be made. Few people in the world have such quality and because of it, they can think up to a level or standard which others cannot do. There is a necessity of such an intelligence and intelligent brain to the human society but the intelligence gets lost after the death of the body and virtual brain is the solution for all these.

Everyone is busy in their lives that they have the difficulty in remembering the events like historical facts, important dates and much more. Availing a machine called the virtual brain is a complete solution for all these issues and relaxes the people without any burden.

V. STEPS FOR BUILDING A BLUE BRAIN

1) Data collection
2) Data simulation
3) Visualization

VI. DATA COLLECTION

It involves collecting brain portions, taking them under a microscope, and gauging the shape and electrical behavior of neurons individually. This method of studying and cataloguing neurons is very familiar and worldwide. The neurons are captured by their shape, electrical and physiological activity, site within the cerebral cortex, and their population density. These observations are translated into precise algorithms which describe the process, function, and positioning methods of neurons. Then, the algorithms are used to generate biologically-real looking virtual neurons ready for simulation.

VII. DATA SIMULATION

It concerns with two major aspects:

1) Simulation speed
2) Simulation workflow Simulation speed Simulations of one cortical column (more than 10,100 neurons) run about two hundred times slower than real time. It takes about five minutes to complete one second of stimulated time. The simulations display unevenly line scaling. Presently the major seek is biological soundness rather than presentation. After understanding biologically significant factors for a given effect it might be feasible to crop constituents that don't subsidize in order to advance performance. Simulation overflow making virtual cells using the algorithms, written to define and describe real neurons, is the major seek of this step. Algorithms and constraints are adapted according to the age, species, and disease stage of the animal being simulated. Each one of the protein is simulated. Note: there are hundreds of millions of proteins in one cell.

VIII. BBP-SDK

The Blue Brain Project - Software Development Kit, a set of Application Programming Interfaces allows the researchers to use and audit prototypes and simulations. The Blue Brain Project SDK is a C++ library wrapped in Java and Python. The primary software used by this for neural simulations is NEURON. Michael Hines of Yale University and John Moore at Duke University developed this in the starting of the 1990s. It uses C, C++, and FORTRAN. It is freely available open source software. The website makes everything available including the code and the binary data freely. Michael Hines in cooperation with BBP team in 2005 ported the package into the massive and parallel Blue Gene.

IX. VISUALIZATION OF RESULTS

RT Neuron RT Neuron is the main application that Blue Brain Project uses for visualization of neural simulations. The BBP team developed this software internally. It is coded using C++ and OpenGL. RT Neuron is ad-hoc software written specifically for neural simulations, i.e. it can't generalize to other kinds of simulation.

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RT Neuron takes the output from Hodgkin-Huxley simulations as input in NEURON and delivers them in 3D. This allows the programmers and researchers to view as activation potentials propagate through or between neurons. The animations can be paused, stopped, started and zoomed, hence allowing the researchers to interact with the model. The visualizations are multi-scale.

X. UPLOADING HUMAN BRAIN

The uploading is possible by the use of small robots known as the Nanobots. These robots are small enough to travel throughout our circulatory system. Traveling into the spine and brain, they will be able to monitor the activity and structure of our central nervous system. They will be able to provide an interface with computers that is as close as our mind can be while we still reside in our biological form. Nanobots could also carefully scan the structure of our brain, providing a complete readout of the connections. This information, when entered into a computer, could then continue to function as us. Thus the data stored in the entire brain will be uploaded into the computer.

XI. ADVANTAGES OF BLUE BRAIN

1) Gathering and Testing 100 Years of Data.
2) The blue brain can remember the things with less effort.
3) The blue brain is an easy way to store and use human intelligence and data or information present in the mind even after the death of the body.
4) It can do all important functions like an intelligent machine.
5) A Novel Tool for Drug Discovery for Brain Disorders

XII. DISADVANTAGES OF BLUE BRAIN

1) It increases the risk of human dependency on Blue Brain every time.
2) Once a Blue Brain related to a particular person's neural schema is hacked, the brain could be used against the person.
3) Another fear is about human clothing and regaining the memory back is an expensive procedure.

XIII. CONCLUSIONS

In conclusion, we will be able to transfer ourselves into computers at some point. Most arguments against this outcome are seemingly easy to circumvent. They are either simple minded, or simply require further time for technology to increase. The only serious threats raised are also overcome as we note the combination of biological and digital technologies.
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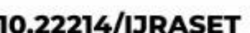

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Embedded system-based intelligent wheelchairs for disabled people

Sanket Sujay Shinde[1], Gurudas Ramdas Mali[2]

[1]Sanket Sujay Shinde, MCA, YTC Satara
[2]Gurudas Ramdas Mali, MCA, YTC Satara
[3] Asst.prof.V.V.Kadam, Asst.prof.S.S.Jadhav, Dept. of MCA, Yashoda Technical Campus Satara, Maharashtra, India

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Abstract - Mobility impairment is a major problem affecting the independence of people with physical disabilities. Therefore, mobility aids need to be improved in order to improve people's living standards. This paper describes the design of smart wheelchairs using embedded systems. This paper describes the design and development of smart wheelchairs using embedded systems. The proposed design of the wheelchair can be controlled via Bluetooth, thus allowing the user to control the wheelchair with less effort. In addition to the virtual joystick control interface, touch commands are provided to the system to enhance user interaction. It helps people with disabilities to carry out their daily indoor activities independently. Experiments will be conducted to verify the functions of the developed smart wheelchair.

1. INTRODUCTION

This paper focuses on the problem of disabled people who want to commute by themselves but cannot drive for natural reasons. This proposed project focuses on Bluetooth control of a wheelchair with automatic movement in directions such as forward, backward, left, right, and diagonal by Bluetooth commands. This model uses an Android app to pass Bluetooth commands to the Raspberry PI 3 via Bluetooth communication with the Bluetooth module. People who become disabled face many problems when moving from one place to another. Most disabled people use conventional wheelchairs. Previously, wheelchairs were manually operated. Operated by hand or by another person if the patient is unable to drive. For this type of wheelchair, the person must have sufficient strength to control it. Otherwise, another person must be present to monitor the movement of the chair. Some face big problems. In this case, a second person is always required [1]. So people working small parts of the body can use it with minimum effort and maximum precision and speed control. The device is loaded with many extra features that make it smart. This wheelchair is therefore designed to overcome the above problems and allow the end-user to perform only safe movements and perform daily necessities.

This paper describes a simple, intelligent, affordable, motor-controlled key device that is easy to use, provides customized commands to the , and allows the wheelchair to move independently. I'm here. A smart phone is used as the robot's brain to give instructions. Bluetooth simplifies his communication from wired to wireless. The IR sensor is also used to detect and notify you when you find an obstacle in your passageway. This design requires the user to control the movement of her wheelchair using Bluetooth commands. These commands are received by the Android application on the user's phone, which is connected to the wheelchair via the Bluetooth module. Commands issued relate to the and RS channels and are received by the module. The purpose of the Bluetooth controlled wheelchair is to listen to the and respond to commands received from the user. This application is just an artificial intelligence application. Here, the system requires training of the user, after which the device will start capturing his commands issued. This is done by attaching comment to the controller via code.

2. Working Methodology

The Smart Wheelchair consists of a wheelchair-controlled Bluetooth module. To set up a system for cheap monitoring, the Raspberry PI 3 UNO allows you to approach the system without viewing the unit. Wheelchair movement can be controlled manually via Bluetooth. Commands are implemented using a Bluetooth mobile app and sent to the Raspberry PI 3 UNO where the commands are processed. [3,4] After processing, the commands are sent in the form of digital signals to the motor driver IC to control it. wheelchair movement. This system was also developed to control wheelchairs with Android devices. The user can steer the wheelchair by selecting specific directions displayed in the four quadrants of the Android smartphone screen. Raspberry PI 3 UNO will try to execute every command. Motor drivers and Bluetooth modules work with this system. This is how you have a car chair that you can drive with your Android device. This project uses a Bluetooth module, namely HC-05. When a button is pressed in the Bluetooth app, the Bluetooth module recognizes the corresponding button pressed in Bluetooth. Your Android smartphone app runs on a smartphone and uses drawing commands recognized by the smartphone's Bluetooth. The command is processed and each transliteration key pressed is executed inside the Bluetooth module and sent through the Bluetooth app to the receiver which is a Raspberry PI 3.This project is controlled by a Raspberry PI 3. The Raspberry PI 3 used is the Raspberry PI 3 UNO. The Raspberry PI 3 UNO is a root development board for the ATmega328 microcontroller with 6 analog

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input means and 14 digital I/O pins. It has 32 KB of flash memory (ISP), 2 KB of RAM, and 1 KB of EPROM. This board offers the possibility of serial communication via UART, SPI. It can operate at a clock frequency of 16MHz. In this project, 2, 3, 4, and 65 are digital input/output pins configured as output pins by the Raspberry PI 3. Serial communication uses 0's and 1's on the HC 05 module. Text received via Bluetooth is sent to the Raspberry PI 3 Uno board using the UART serial communication protocol. The Raspberry PI 3 UNO program controller checks if the received letters match the order of the letters and does not control the movement of the wheelchair at the start.

Table 1. Bluetooth-Controlled Wheelchair Directions

Character	Direction	Function
N	North	Moves Forward
E	East	Moves Right
W	West	Moves Left
S	South	Moves Backward
0	Stop	Motor Stops
1	North-West	Moves Forward Left
3	North-East	Moves Forward Right
7	South-west	Moves Backward Left
9	South-East	Moves Backward Left

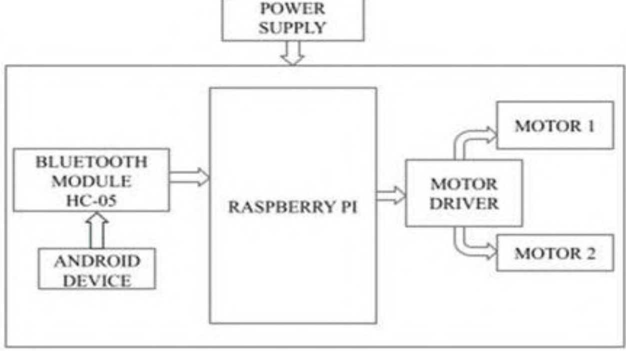

Fig. 1. Block Diagram of Bluetooth controlled Wheelchair

The power supply of this entire system is given through a battery. This system's hardware consists of an embedded system based on the Raspberry Pi 3b, Bluetooth module (HC-05), Motor driver (L298N), Android device and dc motors. Bluetooth Module is the communication medium between the user interface through the android device and the system by means of character commands given to the android device.

All the software application program is installed in the Raspberry Pi and android device. When the user opts for the desired character command through the bluetooth app through android device, then this is transferred to the Raspberry Pi using bluetooth module which is the transmission unit to the receiving unit. According to the desired character opted by the user, the Raspberry Pi gives the signal to the Motor driver as per the algorithm used in the software program. Motor driver gets signal using serial communication and moves the wheelchair either forward, backward or stop. The Android application is connected to the Bluetooth module HC-05 and is installed on the wheelchair by Bluetooth. The commands are sent to the wheelchair using Bluetooth commands present on the Android application. The transmitter of the Bluetooth can take Bluetooth commands which are converted to encoded digital data for the advantage of the adequate 100 meters range from the wheelchair. The receiver part will decode the data via motor driver IC for the necessary commands.

3. Results and Discussions

The Raspberry PI 3 program checks the code received by the Bluetooth module and if it is a matching character with a given program, then with each respective character the Raspberry PI 3 sends a signal to the motor driver. Depending on the received character the Motor. This Bluetooth-module is simple to interface the wireless connection devices. Now the main crucial component of the wheelchair is the L293D Motor Driver. It is a dual H Bridge high current motor driver IC. This is used because digital pins of Raspberry PI 3 can't source enough current to run the motor of a wheelchair.[5] H-bridges are useful in controlling the direction of rotation of the motor enable pins of the IC being actively high are connected to the 5

volts. 4 output pins of l293d IC are connected to Motors A and B at the receiver end.

3.1 Simulation Results

Used Software and application: Proteus 8 Professional Simulation Software, Bluetooth Joystick App

Fig. 3. Motors moves in forward direction as the joystick is in forward direction

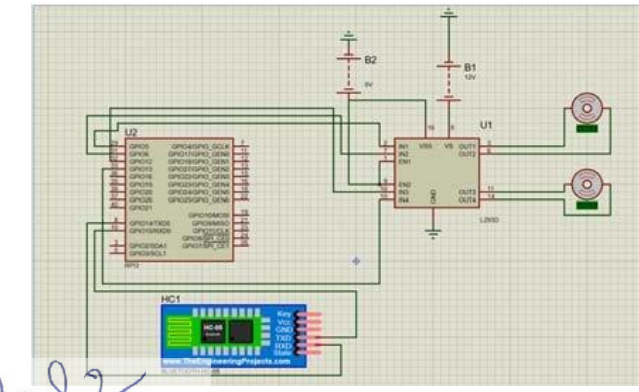

Table 2. Rotation of motors with inputs

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Inputs	Rotation of motors with inputs according to user commands
Input 1 = HIGH	Motor 1 rotates in clockwise direction
Input 2 = LOW	
Input 3 = HIGH	Motor 2 rotates in clockwise direction
Input 4 = LOW	
Input 1 = LOW	Motor 1 rotates in anti-clockwise direction
Input 2 = HIGH	
Input 3 = LOW	Motor 2 rotates in amti-clockwise direction
Input 4 = HIGH	
Input 1 = HIGH	Motor 1 stays still
Input 2 = HIGH	
Input 3 = HIGH	Motor 2 stays still
Input 4 = HIGH	

The wheelchair moves forward when it is instructed to do so by the movement of both the wheels in clockwise direction. It moves backward when both the wheels are given input to move in an anti-clockwise direction. It turns right when the left wheel only turns clockwise and moves left when the right wheel turns clockwise. It moves diagonally forward to the right when the left wheel initially turns clockwise and after a delay, the right wheel also starts turning clockwise. It moves diagonally forward to the left when the right wheel initially turns clockwise and after a delay, the left wheel also starts turning clockwise. It moves diagonally backward to the right when the left wheel initially turns anticlockwise followed by the right wheel after a delay. It moves diagonally backward to the left when the right wheel initially turns anticlockwise followed by the left wheel after a delay.

4. Conclusion

The Present Bluetooth-controlled wheelchair gives a safety and staunch system. It provides a comfortably getat-able and different varieties of functions. In this, we developed a wheelchair that includes ultrasonic sensors to smart track the paths and can detect the objects in the middle of the path along with an ability of maintaining good care to refrain from tragedy, good results are obtained. Hence, paretic peoples can be reliable-self, safely, and ease controlling with this wheelchair. Future changes can improve the wheelchair more. The presented wheelchair will be done by appending new advanced sensors, in order to make the wheelchair increased friendliness and to refrain from tragedy by learning-self. Security will be integrated for retrieval with the assistance.

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Mobility operation in the 5G Network between colorful Access Networks

Dipali Shankar Shinde[1], Pratiksha Narayan Kadam[2], Asst.prof.S.S.Jadhav[3], Asst.prof.V.V.Kadam[4]

[1]Pratiksha Narayan Kadam, MCA, YTC Satara
[2]Dipali Shankar Shinde, MCA, YTC Satara
[3]Asst.Prof.S.S.Jadhav,[4]Asst.prof.V.V.Kadam Dept. of MCA, Yashoda Technical Campus Satara, Maharashtra, India

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Abstract— The 5G network, which aims to manipulate in 2020, is secerning in terms of data transmission speed, quiescence, and capacity of outstations on the network compared with the 4G network. One of the major design generalities for the 5G network is to accommodate colorful multiple access networks with the core network, and to give flawless mobility service. In this paper, we present the conception of Multiple Access Protocol Data Unit(MAPDU) session to control large data transmission in 5G network, and propose a dynamic anchoring mobility operation between different access networks.

Keywords—dynamic anchoring, mobility management, 5G network

1. INTRODUCTION

The 5G network, which aims to manipulate in 2020, differs from the 4G network in terms of data transmission speed, quiescence, and capacity of outstations on the network. In addition, the 5G network is anticipated to play a part not only as an structure for mobile communication services, but also as a base for future diligence

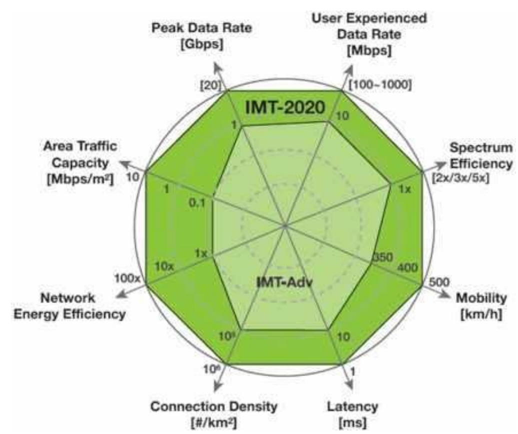

Fig. 1. Enhancement of key capabilities from 4G to 5G

The 5G network aims to achieve data rates of over to 20 Gbps, which is 20 times faster than the 4G network with a outside of 1 Gbps(2). still, the factual data transmission speed that 5G mobile service druggies can witness is aimed at 100 Mbps. This raises enterprises about whether it'll be possible to handle contents that bear large quantities of data, similar as virtual reality or holograms. To break this problem, one of the major design generalities for the 5G network is to accommodate colorful multiple access networks with the core network. This allows druggies to enjoy immersive contents that they hadn't preliminarily endured through the 5G network. For this purpose, there's a need for a control system able of transmitting a large quantum of data by contemporaneously using colorful kinds of access networks constituting the 5G network(6).

Another of the main design generalities for 5G network is to have a distributed control structure to help centralization of data business. The 4G network has a hierarchical structure in which several S- GWs are connected to a P- GW where an IP address is anchored and several base stations are connected to the S- GW. thus, in order to use the Internet service, the data business is concentrated in the P- GW, performing in hamstrung data paths. In the 5G network, the GW that anchoring the IP address is distributed close to the access networks to support a large quantum of data business. In order to support similar distributed structure, mobility control for data business between anchoring GWs is needed. While the stoner terminal moves in the 5G network and coincidently attaches to the 3GPP and the Non3GPP Access networks, and when the data packet transmitted to the 3GPP Access network is path switched to theNon-3GPP Access network, there may arise a problem that order of the packets isn't guaranteed because of the transmission detention difference on the paths in the colorful access networks.

In this paper, we present the conception of Mama- PDU(Multi Access PDU) session to control large data transmission in 5G network, and propose mobility control system to guarantee nonstop data transmission between multiple access networks. The remainder of this paper is organized as follows. In Section 2, affiliated exploration trends on mobility control are explained. Section 3 describes the structure of 5G network, which is being formalized in 3GPP. In Section 4, a dynamic anchoring mobility operation with the End Marker is presented to insure flawless data transmission between 5G and WiFi access networks

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2. RELATED WORKS

In the 4G network as in the figure 2, 3 realities similar as the Serving Gateway(S- GW), the Packet Data Network Gateway(P- GW), and the Mobility operation reality(MME), manage mobility functions. The point of the 4G network, in which all the business generated by the outstation is transmitted to the central P- GW due to mobility operation and billing, causes hamstrung business paths. In addition, 4G network doesn't give mobility control between the anchoring GWs. Since there isn't a unified mobility operation between colorful access networks and LTE access and WiFi access networks operate independently(3).

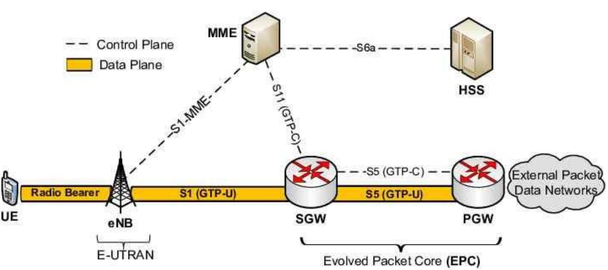
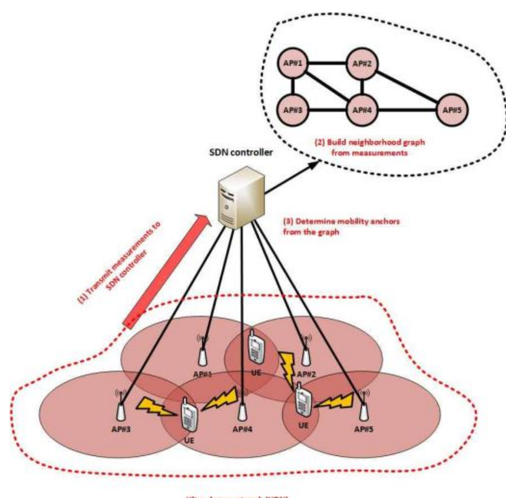


Fig. 2. 4G Network Architecture

The 5G network being formalized in 3GPP provides the conception of Demand on Mobility. The mobility characteristic and the mobility position are classified according to the types of the terminal, similar as a detector in a stationary state or a vehicle under operation, thereby minimizing the paging signaling between the UE and the core network. As the UE moves through a lot of stoner Aeroplane Functions(UPFs), the problem about Session and Service Continuity(SSC) has been considered. Indeed though three ways are defined to break the problem, but procedures for furnishing mobility operation in colorful access networks aren't defined in detail yet.



`Fig. 3. Overall steps of centrality based SDN- DMM

Proposes an optimization of the handover interruption time in the SDN grounded distributed mobility operation(DMM). The idea of this paper is to use centrality for ranking the bumps of the network. The bumps with the loftiest centrality are named as mobility anchors for the data and control aeroplane of the stoner outstations of the network. But, since this offer is grounded on SDN regulator, the 5G network armature in 3GPP isn't reflected.

Proposes a network armature that employs MMEs as a logical function in the mobile network. These MMEs perform UE operation autonomously in a distributed manner as independent distributed MME(ADMME). The main thing is to propose a new network armature that solves problems in the ADMME selection system while retaining its advantages. still, this offer is still grounded on 4G network and adds an reality to manage mobility in the RAN.

Analyzes stoner and control aeroplane quiescence, handover prosecution time, and content of functional LTE networks. This paper explains that the LTE handover prosecution time conditions and observed performance are analogous. Since the connected mobility use cases are targeting safety and effectiveness bear zero service interruption time, it suggests that the 5G design must use new mobility styles similar as makebefore- break andmulti-cell-connectivity.

3. 5G NETWORK ARCHITECTURE

A. Design Principles

The 5G network armature has been defined in 3GPP to support data connectivity service. The 5G network armature uses service- grounded relations between Control Plane Network Functions where linked. Some crucial principles and conception are as follows

-Separate the stoner Aeroplane(UP) functions from the Control Aeroplane(CP) functions, allowing independent scalability, elaboration and flexible deployments.

- Minimize dependences between the Access Network(AN) and the Core Network(CN). The armature is defined with a gathered core network with a common AN- CN interface which integrates different Access Types(e.g. 3GPP access andnon-3GPP access).

- Modularize the function design(e.g. to enable flexible and effective network slicing).

- Support concurrent access to original and centralized services. stoner Aeroplane functions can be stationed near to the Access Network to support low quiescence services and access to original data networks.

B. Architecture Reference Model

The 5G network armature as in the figure 4 consists of the following network functions(NF).

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- Access and Mobility operation Function(AMF) AMF provides functionalities similar as Termination of RAN CP NAS, Registration/ Reachability/ Mobility operation, Access Authentication/ Authorization

- Session operation Function(SMF) SMF provides functionalities similar as session operation, UE IP address allocation, control of UPF, termination of SM corridor of NAS dispatches.

-stoner Aeroplane Function(UPF) UPF provides functionalities similar as Anchor point for mobility, Packet routing & forwarding, QoS handling

- Authentication Garçon Function(AUSF)

-operation Function(AF)

- Data Network(DN)e.g. Internet access, 3rd party services, driver services

- Network Slice Selection Function(NSSF)

- Policy Control Function(PCF)

- Unified Data Management(UDM)

- stoner outfit(UE)

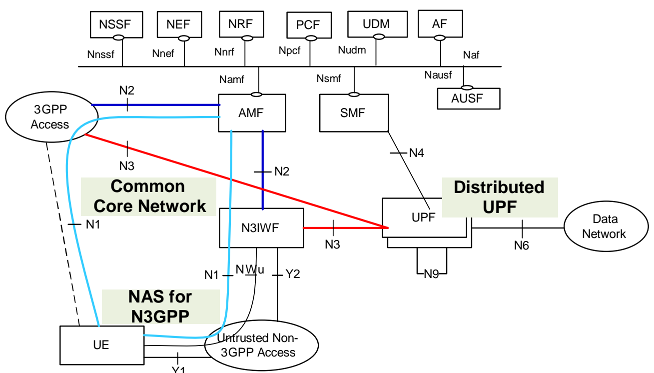

Fig. 4. 5G Network Architecture

4. MOBILITY MANAGEMENT IN THE 5G NETWOK

In order to break the problem of the mobility operation in the 4G network, dynamic anchoring mobility control, in which the anchoring GW for managing the IP address of the UE is changed as the UE moves, is being introduced in the 5G network(5).

In this section, we consider a 5G network where a UE can use 5G access and WiFi access contemporaneously as shown in Fig 5. We propose a mobility operation grounded on dynamic anchoring with the End Marker that guarantees the durability of sessions when the UE moves between colorful access networks belonging to different anchoring GWs. To do this, the UE must be suitable to control the Mama- PDU session, and the 5G core network must supportinter-GW Handover discovery of multi RATs and mobility operation for on- going PDU sessions.

A. Support for MA- PDU Session-

The 5G Network supports a PDU(Protocol Data Unit) Connectivity Service i.e. a service that provides exchange of PDUs between a UE and a data network linked by a DNN(Data Network Name). The PDU Connectivity Service is supported via PDU Sessions that are established upon request from the UE(5).

A Mama- PDU(Multi Access PDU) is a type of PDU session that allows operation to shoot admit business either over 3GPP access, ornon-3GPP access, or both accesses contemporaneously. A Mama- PDU session comprises of a PDU session over 3GPP access and a PDU session overnon-3GPP access, or vice versa. Each of the PDU sessions may have its own set of UPFs, but both PDU sessions partake a common PDU session anchor(PSA). A Mama- PDU session is created by speeding together two separate PDU sessions, which are established over different accesses.

B. UE Architecture for MA- PDU Session-

During MA- PDU Session Establishment procedure, the SMF sends the IP address to the UE via SM NAS signalling. To control the Mama- PDU session and theInter-GW Handover function, the UE supports Logical Interface(LIF) to acclimatize given IP address to inner IP addresses for multi access interfaces independently. Figure 7 shows the armature of a UE with a LIF to support multiple inner IP addresses for colorful access types.

The LIFs in the link subcaste of the UE may have different inner IP addresses, and each LIF is created one by one whenever a sub PDU session is created. The Mama- PDU session is generated through NAS signaling between the UECB(stoner Equipment Control Block), which processes the NAS dispatches at the UE, and the AMF. The UECB transmits the IP address, which is allocated at the time of Mama- PDU session creation, and the UPF information to the LICB(Logical Interface Control Block) which manages the logical interface. The LICB generates the LIF using the corresponding information, and sets up the routing table for mapping the business of each sub PDU session to the corresponding LIF.

The LIF generation and business control procedures in the UE, when the UE performs theinter-GW handover(HO), are as follows.

Previous to the handover of the UE, an IP# 1 address is allocated to the LIF# 1 according to the NAS procedure, and the IP address of the source UPF is set as the dereliction anchoring gateway in the routing table of the UE.

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According to the routing policy of the UE kernel, the operation of the UE transmits and receives data using the IP# 1 address. After the UE performsinter-GW handover, it changes the On- going Session from the source UPF to the target UPF according to the NAS procedure, and the target UPF and the target base station(BS) establish a lair for recycling the business of the On- going Session. The operation of the UE transmits and receives the data using the being IP# 1 address

C. Support for Dynamic Anchoring Handover

While the UE moves between different access networks in the 5G network, theinter-GW(between anchoring UPFs) mobility operation grounded on Dynamic Anchoring, which guarantees the packet transmission order through the End Marker exchange, is as follows.

When there's a session created in the source UPF, the mobility control procedure of the on- going session is different depending on whether there's an Xn interface(inter-base stations interface) between the source BS and the targetBS. However, a forwarding lair is created between the source BS and the target BS, If there's an Xninterface. However, an circular forwarding lair is created between the source BS and the target BS, If there's no Xn interface. The circular forwarding lair is formed from the source BS to the target BS through the source UPF and the target UPF.

1)Inter-GW Handover procedure for On- going Session with Xn Interface

The Xn groundedInter-GW Handover procedure for on-going session is shown inFig. 8 left side. Source 5G- BS determines handover to target WiFI- BS through dimension control. However, a Handover Prepare procedure is performed through the Xn interface, and a forwarding lair between the source 5G- BS and the target WiFi- BS is created, If there's an Xn interface between the BSs.

The target WiFi- BS buffers packets entered from the forwarding lair until a Handover Confirm communication is entered from the UE. The target WiFi- BS sends the N2 Path Switch Request communication to the AMF, and the AMF sends the corresponding communication to the SMF.

The SMF selects a new target UPF of the UE with reference to the ID of the target WiFi- BS. The SMF generates anInter-GW lair for handover of an on- going session by transferring a session creation and session change dispatches to the target UPF and the source UPF.

The source UPF sends an End Marker to the source 5G- BS at the time of the path change to shoot the packet to the target UPF. The target WiFi- BS first transmits the packet entered through the forwarding lair to the UE until it receives the End Marker. The target WiFi- BS buffers the packet entered through theInter-GW lair until it receives the End Marker, and transmits it to the UE after entering the End Marker.

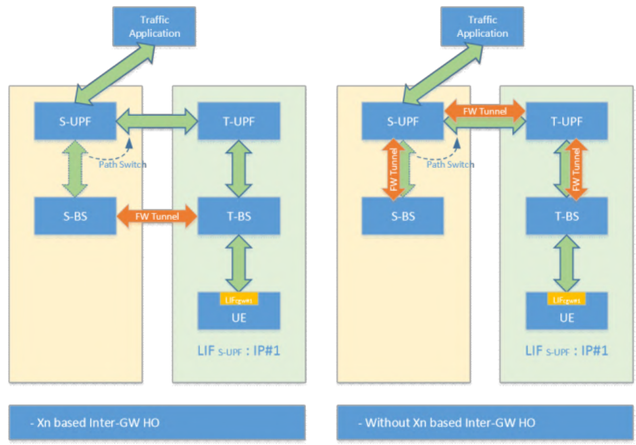

Fig. 5. Inter-GW Handover procedure for On-going Session

2)Inter-GW Handover procedure for On- going Session without Xn Interface

Theinter-GW Handover procedure in the absence of an Xn interface between different access BSs is shown inFig. 8 right side. The source 5G- BS determines the handover to the target WiFi- BS through the dimension control and notifies the target WiFi- BS of the handover medication procedure through AMF and SMF.

The SMF generates an circular forwarding lair through the source 5G- BS- source UPF- target UPF- target WiFi- BS.

Until the UE completes the L2 handover, the packets entered by the source 5G- BS are encouraged to the target WiFi- BS through the circular forwarding lair.

When the L2 handover of the UE is completed, the target WiFi- BS sends a Handover Notify communication to the AMF, and the AMF sends a Handover Complete communication to the SMF through the N11 interface. AMF creates anInter-GW lair to further the packet from the source UPF to the target UPF.

In the path switching step, the source UPF transmits the End Marker to the source 5G- BS. The target WiFi- BS transmits the packet entered through the forwarding lair to the UE until it receives the End Marker. The target WiFi- BS buffers the data packets entered through theInter-GW lair until it receives the End Marker, and transmits the softened data packets to the UE after entering the End Marker.

The Source UPF buffers stoner data business after the Path Switch without transmitting it to theNon-3GPP access network in order to guarantee the order of business packets. The source UPF transmits the last stoner business transmitted to the 3GPP access network with an end marker.

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The 3GPP access network transmits the stoner business, which transmitted from the source anchoring UPF to the UE with the end marker. also, it sends the End Marker to the target anchoring UPF. This guarantees the packet transmission order.

3) Optimizing Paths for New Sessions

After the handover procedure for the on- going session is completed, a new PDU session of the moved UE is created by assigning a new IP address to the changed anchoring UPF. The SMF sends an N11 Acknowledgement communication during a handover procedure for an on- going session which notifies an suggestion for requesting the creation of a new session. The UE starts a new session creation procedure via changed anchoring UPF. Through this procedure, the new session is routed through new UPF, which is the optimal path, without going through the being source anchor UPF.

5. CONCLUSION

The 5G network accommodates colorful access networks similar as 5G, WiFi, and Fixed interfaces under the single control medium in the 5G core network, and attempts to break the vexation of service interruption when UEs move between multiple access networks.

In this paper, we propose a dynamic anchoring mobility operation with the End Marker to guarantee the transmission order of packets when a UE moves in colorful access networks in 5G network. Through the proposed mobility operation, the 5G network provides the optimal network terrain for furnishing a more effective and flawless communication service to druggies

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API Testing Using Postman Tool

Pratik Prakash Kore[1], Mayuresh Jaywant Lohar[2], Madhusudan Tanaji Surve[3], Snehal Jadhav[4]
[1, 2, 3]MCA Department, Shivaji University, Kolhapur
[4]Associate Prof. MCA Department, Yashoda Technical Campus, Satara

Abstract: Postman is one of the greatest API automation and documentation tools available today. Postman began as a simple Chrome browser plugin and has now grown to be a full API testing solution used by 5 million developers and over 100,000 enterprises all over the world. With a $2 billion value, it's a unicorn in its own right, and it's become the go-to platform for creating enterprise APIs.
As the API economy continues to growing, more challenges are created for developers. The old methods of manually creating and testing APIs no longtime scale as today's software and services can interface with hundreds of APIs within a single application. Development, testing, and delivery teams must work together to make sure that applications work barrier with APIs to provide a business advantage, rather than cause a business obstacle.
Collaboration and operational efficiency are the keys to sharing modern API- powered applications. And this is the space that Postman plays in. In one of our GlobalLogic projects related to a Banking as well as Financial Services Customer Communications Management platform that is CCMP, we need to use Postman and its CI/CD add-on Newman to create, customize and automate Web API tests. This white paper and technical report narrate our passage direct this automation and showcases some learnings and best practices realized along the way. This is our view from the trenches on the capacity and possibilities of the Postman platform for API testing.
Keywords: Connecting to API, Postman Tool

I. INTRODUCTION

API is a Application Programming Interface. They are connection Between two Application. In API There are two types one types is use for without internet connection and another type is used for with internet connection. Those types one is used with internet connection is call 'Web Services'.
Let see What is web services?
For Example: You took a Flipkart of application, Flipkart is a mobile app and as well a web app. You can use Flipkart whenever your internet you can't turn on Flipkart. The data you will using the Flipkart application the data will be connected to your server. web application you use to connect to the data server is also called API. API is divide inti many types but there are two main method currently used First is SOAP Method and Another is REST Method. The SOAP Method is doesn't use much because Longform of SOAP is Simple Object Access Protocol. If you want to connect through SOAP API then you can use xml body and SOAP API is only use only POST API Method. They are in return xml body, because they are heavy, they consuming a more bandwidth and it is a Slow. In REST API we can use xml, plan-text format. Every format gives different method like POST,GET, PUT, DELETE different method are available for each operation. They are lightweight and they are fast comparatively SOAP API. Because of all this reasons most people use REST API service.

II. NEED OF WEB SCRAPING

Postman is an API client for developers that makes it simple to create, distribute, test, and document APIs. Users may construct and save basic and sophisticated HTTP/s queries, as well as view their answers, to do this. As a result, work is more efficient and less tiresome.

III.PROPERTIES OF REST API

1) REST API follows the client -Server Architecture.
2) Stateless
3) Cache
4) Uniform Interface

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REST API is used for connection between Client and Server. User used is client and your coding and logic is save is server. So the REST API is establish the relat=-09on between Client and Server.

In above diagram Flipkart is a client. You can open Flipkart.com website if you send the request to server and display the server response.

REST- Representational State Transfer. REST API connection between client and server. They follow client server relationship. They can Follow client-server architecture.

Stateless- In above diagram, client is connected to server first time and server give a response to client request. Then client send request to server second time they are totally unknown about first request and response. Server never save any data to him. No any data save about request to server. Because they called stateless. Stateless means server does not store any session data. Server do not store any session related data with it.

Cache- The cache is present in API Application for storing a system and retrieve a request network and their response .the memory of cache is important because they are improve data retrieval. In

CPU cache is you found primary cache memory. secondary memory is found in a separate clip close to the CPU.

Uniform Interface-In uniform Interface, Server is connect browser through or server is connected to mobile through 890- 0987/Two application are connected but the layer or interface used to connect service through browser or mobile application is common. If you fire the request through mobile or through browser in backend create the REST API are uniform means Common API's are created So, that is the Uniform Interface.

IV. REST REQUEST

A. *Method Type*

When a client is communicate with the server it needs to indicate what kind of action that it experts that particular request is from of HTTP request method. We will see the different method type. In Method types includes GET, PUT, POST, DELETE etc.

Actual work of this method let see one example we take a user. User can create, edit, read, or delete the data. This Create, Edit, Read and Delete are known as the CRUD Operations. We can perform CRUD operation on data. In CRUD Operation, C for Create, R for Read, U for Update, and D for Delete. In Method Type suppose you can send the request for server, you need to say the which request you can send and what perform this request. There are so many method types are their but the four important method.

Create-POST Read- GET Update-PUT Delete- DELETE

In short, Method type means which operations perform going on REST API is understand in method type.

B. *Endpoint*

Suppose, You have a four environment, You can understand these In four request where your request is goes. If you execute your query on development server then the endpoint of URL is Development server. Same as you can execute same query on QA server then endpoint of URL is QA server.

Example: www.googledev.co.in/complete . if you execute this request first time you can find first request is goes through which server. It is depend on endpoint or path parameter.

C. *Path Parameter/Query Parameter*

In path parameter is a Additional information is sent to the server via request parameters. These parameters are contained in a URL.

Query parameters are appended to the end of the request URL, following '?' and listed in key-value pairs, separated by '&' Syntax:

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Query parameters are appended to the end of the request URL, following '?' and listed in key-value pairs, separated by '&' Syntax:

?id=1&type=new

Path parameters are part of the request URL and are accessible using placeholders followed by ':'. Example: /customer/:id

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D. Headers

HTTP request or response. The headers are shown in Postman's Headers tab.

When you click on the header, you'll get a variety of information, such as the one below. Despite the fact that every element on the Headers tab is a header item, we'll just look at the most significant ones.

1) Content-Type
2) Date
3) Server
4) Cookie expire time

V. CONCLUSIONS

Hence Postman is a sophisticated, powerful, and versatile API testing software. Having a fully flexible continuous testing infrastructure for APIs is very real with Newman as an add-on. Based on what we learned in our project, we believe that using the Postman toolchain for continuous API testing will help most development projects.

This study looked at how to verify the security of an Application Programming Interface (API)

In a nutshell, the Postman and Swagger tools are silent. These days, it's all the rage in the industry. The majority of the Apps connect with other applications in order to accomplish their goals. Use an API to share and obtain data feeds, whether it's from Google, Facebook, or Twitter. Facebook, smartphone apps, and online apps are all options. We concentrated on RESTful APIs Specifically, the security of RESTful APIs

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Virtual Smart Phones

Rutuja Suryakant Deshmukh[1], Vijaya Mahendra Mohite[2], Prof.Dr.S.P.Jadhav[3], Prof.S.S.Jadhav[4]

[1st]Rutuja Suryakant Deshmukh, MCA YTC, Satara
[2nd]Vijaya Mahendra Mohite, MCA YTC Satara
[3rd] Prof.Dr.S.P.Jadhav,[4th] Prof. S.S. Jadhav Dept. of MCA Yashoda Technical Campus,Satara-415003

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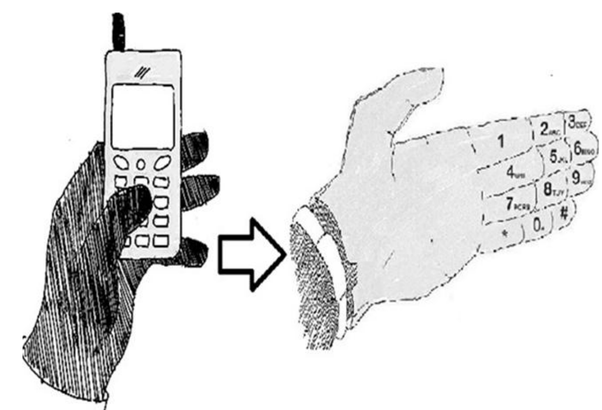
***Abstract*:-** For every departure metal money communication may be a manner by that they share/pass their thoughts/fillings to 1 another. we have a tendency to homosepians chiefly use verbal communication to speak with one another. during this Paper we have a tendency to introduce VSP, a Virtual good Phone that is largely a step to attach each the Physical and virtual world, by employing a little projector, Camera, Speaker, microphone & Cloud Computing Technology over the net within the kind of wearable device. In VSP all the specified element area unit fancied within the wearable device by that use communicate with the assistance of natural hand gesture, Hand movement and net. In VSP user communicate with one another by Virtual mobile with the assistance of bit gesture electromagnetic radiation and cloud computing technology.

VSP can finish the physical dependency of mobile. VSP offer novel interaction methodology to seamlessly communicate with one another in an exceedingly fun and intuitive manner. The user will bit their Palm to form decision and might even be used for looking at movies or pictures on their Palm/wrist. bit gesture is employed for creating and Terminating the decision. VSP uses touch-based interactions as instruction for establishing communication between the various users.

1. INTRODUCTION

The recent advent of novel sensing and show technologies has inspired the event of a spread of multi-touch and gesture primarily based interactive systems. In these systems user could move directly with info victimization bit add natural hand gestures. these days there area unit voluminous approach by that we will hook up with digital world within the controlled surroundings victimization muti-touch and gesture primarily based interaction. sadly, most gestural and multi-touch primarily based interactive systems don't seem to be mobile and little mobile devices fail to supply the intuitive expertise of full-sized gestural systems.

Moreover, info still resides on screens or dedicated projection surfaces. there's no link between our interaction with these digital devices and interaction with the physical world around US. during this paper, we have a tendency to gift VSP-Virtual sensible Phone, a multi-touch and gesture primarily based interaction system. that replace the physical transportable device to the virtual multi-touch & natural gesture primarily based interaction on the user palm by that user communicate with alternative digital devices over the network. VSP primarily turns the human hand as a transportable by that is ready to user hook up with the digital world additionally as alternative peoples like their friends and relatives.



VSP is largely a computer-vision primarily based wearable and gestural info interface that augments the physical world around US with digital info and proposes natural hand gestures because the mechanism to move therewith info.

2. RELATED WORK

Recently, there are an excellent form of multi-touch interaction and mobile device merchandise or analysis prototypes that have created it doable to directly manipulate computer programme parts victimisation bit and natural hand gestures. Most of those systems rely upon the physical bit-based interaction between the user's fingers and physical screen and therefore don't acknowledge and incorporate touch freelance freehanded gestures. VSP Virtual sensible Phone Technology takes a unique approach to computing and tries to form the digital facet of our lives a lot of intuitive, interactive and, above all, a lot of natural. It's plenty of advanced technology squeezed into a straightforward moveable device. once we herald property, we are able to get instant, relevant visual info projected on any object we tend to develop or act with the technology is especially supported hand increased reality, gesture recognition, laptop vision based mostly formula etc.

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Augmented Reality

Augmented reality (AR) may be a term for a live direct or indirect read of a physical globe setting whose components square measure increased by virtual computer-generated imagination. it's associated with a a lot of general idea referred to as mediate reality during which a read of reality is changed (possibly even diminished instead of augmented) by a laptop. The augmentation is conventionally in time period and in linguistics context with environmental components.

Virtual good Phone uses increased Reality idea to position digital info on the physical world. With the assistance of advanced AR technology (e.g. adding laptop vision and object recognition) the data concerning the encompassing globe of the user becomes interactive and digitally usable. Artificial info concerning the setting and therefore the objects in it are often keep associate degreed retrieved as an info layer on high of the important view. the most hardware elements for increased reality are: show, tracking, input devices, and laptop. Combination of powerful hardware, camera, accelerometers, GPS and solid state compass square measure typically gift in fashionable Smartphone, that create them prospective platforms

Gesture Recognition

Gesture recognition may be a topic in engineering science and language technology with the goal of decoding human gestures via mathematical algorithms. Gestures will originate from any bodily motion or state however normally originate from the face or hand. Current focuses within the field embrace feeling recognition from the face and hand gesture recognition. several approaches are created mistreatment cameras and pc vision algorithms to interpret signing. Gesture recognition will be seen as how for computers to start to know soma language, so building a richer bridge between machines and humans than primitive text user interfaces or perhaps GUIs (graphical user interfaces), that still limit the bulk of input to keyboard and mouse. Gesture recognition allows humans to interface with the machine (HMI) and act naturally with none mechanical devices. Gestures will be wont to communicate with a pc thus we are going to be largely involved with empty handed semiotical gestures

Computer vision Based Algorithm

Computer vision is that the science and technology of machines that may see. As a study, laptop vision worries with the idea behind artificial systems that extract info from pictures. The image information will take several forms, like video sequences, views from multiple cameras, or multi-dimensional information from a medical scanner. The software system tracks the user's gestures mistreatment computer vision primarily based algorithms. the pc vision system for pursuit and recognizing the hand postures that management the menus relies on a mixture of multi-scale color feature detection, read primarily based stratified hand models and particle filtering. The hand postures or states ar depicted in terms of hierarchies of multi-scale color image options at totally different scales, with qualitative interrelations in terms of scale, position and orientation. In every image, detection of time period color options is performed.

The hand postures ar then at the same time detected and half-tracked mistreatment particle filtering, with associate degree extension of superimposed sampling stated as stratified superimposed sampling. to boost the performance of the system, a previous on skin colour is enclosed within the particle filtering. Figure 2: Gesture Recognized Mobile keyboard VSP conjointly|is additionally} associated with increased reality wherever digital info is superimposed on the user's read of a scene however it also take issue in many vital ways that. 1st VSP permits user to move with the projected info mistreatment hand gestures. Second the knowledge is projected onto the Hand/object and surfaces themselves, instead of onto glasses, spectacles or watch which ends in a {very} very totally different user expertise.

3. OBJECTIVE

VSP Invention is said to transfer of knowledge & establishing communication from one physical body to different physical body or from one physical body to digital devices or vice-versa with none platform dependency. VSP is essentially AN makes an attempt to create the communication between users and Digital devices additional tangible and interactive. the target of this invention is establishing the connection/communication between humans and conjointly with digital devices by barely gesture on the human Palm/Hand. VSP work on 2 kind of information transfer.

First, It establish auditory communication between the users with the assistance of GSM Technology with none physical cell phone.

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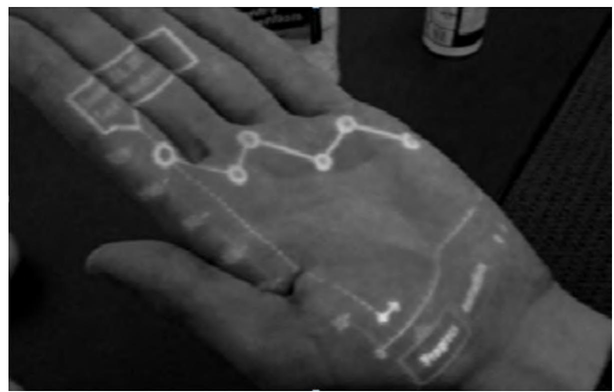
Second, For Transfer of knowledge between the humans and conjointly with digital devices. It create use of the net, computer network network or the other kind of information Servers through that device and humans area unit connected to and also the distinguish from one user to a different by the authentication ways like username/password, drawing a pattern on the virtual screen, face recognition, Palm recognition victimization palm lines or fingerprint detection will be used. In VSP auditory communication type one human to a different will be done either by victimization GSM or Internet/Intranet technology.



The Transferring of knowledge from one creature to a different or device victimization VSP. the primary and second digital devices is also gesture recognition VSP system connected to a network as well as a knowledge storage cloud and each uses VSP Technology.

4. WORKING

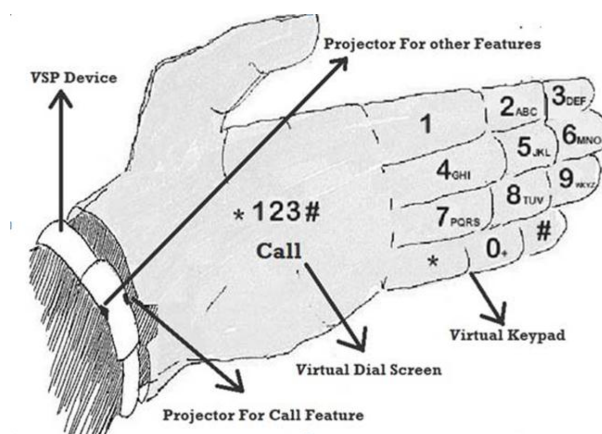
Working of VSP accommodates five Main steps i.e. sanctioning & evidence VSP, Make Call, Receive decision, Capture Image/Video, repetition knowledge & paste/Pass knowledge to different VSP & Digital Devices as follows.

A. sanctioning VSP

The VSP may be a wearable device and user has the key to change (ON)/Disable (OFF) the device through the ability Button. once user change the VSP Device, associate icon seems on the user palm or arm as per user as per designated by the user for showing the standing (if a user has signed in).If not user will bit this icon to login or modification users victimization totally different authentication strategies like: Enter user name and secret, Drawing a secret sign or pattern, Face recognition, image choice and Fingerprint detection and Palm line Detection once a user has signed in with success, VSP is currently prepared for creating and receive calls and different Operations

B. Make Call

After enabling VSP currently user is in a position to create decision and communicate with their relatives and alternative persons. to create decision, Dial mobile range mistreatment virtual key or mistreatment Voice Recognition system. For establishing decision between 2 users, VSP uses 2 technique that ar as follows.

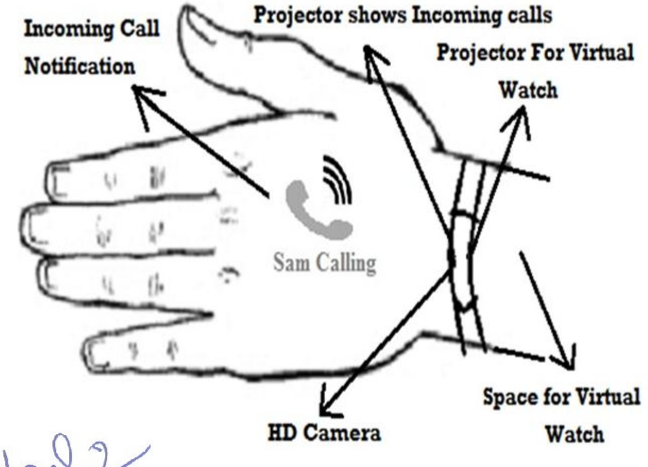


a. build decision mistreatment SIM:

VSP device encompasses a small SIM (Subscriber Identity Module) by that device established the decision mistreatment GSM/CDMA (Global System for Mobile Communications / Code Division Multiple Access) Technology.

b. build decision mistreatment VOIP:

VSP device encompasses a Wi-Fi (Wireless Fidelity) and Mobile information possibility that connect the device to the Intranet/Internet, by mistreatment this user is in a position to create calls mistreatment VOIP (Voice Over IP) Technology. By mistreatment VOIP user is in a position to create the decision to alternative VSP user additionally as all the others GSM and net VOIP change Digital Devices. once user isn't connected to internet/Intranet, decision is just created mistreatment SIM while not user's permission however once user hook up with net it raise user to pick the choice by that user needed to create decision as per user choice the decision is hook up with alternative person.



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C. Receive decision

When a VSP user referred to as by different VSP user or different digital device users by (Physical portable laptop computer, Desktop and personal organiser 'Personal knowledge assistant) the notification of incoming decision are going to be shown as per user designated Profile if user choose vibrate mode, the tiny vibrator motor indicate incoming decision by vibration & conjointly shows the identity of occupation user on back aspect of palm victimization high Density projector of VSP. If user choose Sound Mode, incoming decision notified by designated ring tone with user Name on the rear aspect of Palm. In Silent mode it solely indicate the name of caller within the back aspect of palm. For attending the incoming decision user simply bit, swipe the incoming decision icon or different bit gesture designated by user. to talk the caller user either use Bluetooth telephone receiver or wired telephone receiver that is connected to VSP device victimization three.0 connective. User is also ready to receive decision directly victimization VSP Device Speaker and Mice. For VOIP calls each user should be connected to the net victimization WI-FI or Mobile knowledge.

D. Capture Image/Video

VSP is additionally ready to capture prime quality Images/Video victimization their prime quality Camera by click capture image button or by victimization gesture (make a fame victimization our index figure and thumbs) for taking photos. once taking the image it shows the image on user hand victimization VSP System. For shoot video with constant gesture user simply needed to alter the camera mode photos to video. User conjointly center or zoom out whereas they capture Image/Video victimization their hand gesture.

E. Copy Data:

In VSP permit users to Transfer (Copy/Paste) knowledge from one shape to a different shape or device by employing a single bit gesture. For copy knowledge user must login initial in VSP device and connected to Internet/Intranet. For distinctive a duplicate event in VSP uses an extended press (Detect by perceiver Program) on copy ready knowledge item (keeping finger on a knowledge item quite one.5 sec. shown on user arm victimization VSP projector) indicates to repeat that knowledge item. Whenever user bit any copy ready knowledge barely perceiver program begin investigating the time and once time exceeds the edge (1.5 sec.) a message seems indicating that {the knowledge|the info|the information} item is being traced and gets traced to the user's distinctive area within the data cloud. The copy knowledge to the information cloud may also be done by other ways (instead of long-press for one.5 seconds). for instance, double faucet on knowledge item or draw a circle the information item to initiate copy. victimization this method user copy multiple file for passing/paste to the opposite device all the copy knowledge save within the cloud on temporary bases with distinctive id of every knowledge item.

5. TECHNOLOGIES USED

VSP is largely a wearable device that is combination of hardware still as software system. In hardware VSP incorporates Processor Unit, Ram & storage Memory, Power provide (battery), Sensors (Accelerometer, sixteen Proximity sensing element for distinguishing bit on Arm), light-emitting diode Indicator For Device Mode (ON/OFF), small Vibrator Motor, USB port (For charging or attaching different devices), four small Projectors (like Pico Projectors), one HD Camera for Capturing pictures and videos, Low energy needed WI-FI and Bluetooth devices, GPS system, four bit buttons (ON/OFF Button, Snap Button, sound Up button, sound down button) and Nano SIM card slot. In software system it use gesture recognition system, bit based mostly interaction system, increased Reality, laptop vision based mostly formula to meet all the objectives.

VSP uses the subsequent Technology for create decision, Receive decision, repetition knowledge & paste/Pass knowledge to different VSP & Digital Devices.

a. Voice Call:

In VSP voice decision done by exploitation either by exploitation SIM (GSM/CDMA) or although net exploitation VOIP Technology.

b. knowledge Transfer:

Data transfer from one body to a different body or device in exploitation VSP is completed by exploitation knowledge Cloud. For Accessing knowledge cloud user is also connected to net either by WI-FI or Mobile knowledge exploitation SIM.

6. CONCLUSION

VSP is essentially a computer-vision primarily based wearable and gestural interface that augments the physical world around United States with digital info and proposes natural hand gestures because the mechanism to move thereupon info. It connect Physical world to Virtual world. VSP provide intuitive thanks to communicate and knowledge Transfer between completely different|completely different} users similarly as different Digital Devices.

VSP invention fulfill our 2 future necessities. First, it's free morpheme physical dependencies of devices. Second, it connect our physical world to virtual world Some Application of VSP as Follows:

1. employed in Health watching System.

2. accustomed realize info of any Product/Item.

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3. accustomed Connect News and Weather Update.

4. accustomed connect completely different Devices just about.

5. employed in Education & coaching system.

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Secure Desktop Computing In the Cloud

Priyanka Shankar Bhingardeve[1], Prajkta Mahendra Ghadge[2], Asst.Dr.S.P.Jadhav[3], Asst.prof.S.V.Thorat[4]

[1st]Priyanka Shankar Bhingardeve, MCA YTC, Satara
[2nd]Prajkta Mahendra Ghadge, MCA YTC Satara
[3rd]Dr.S.P.Jadhav,[4th]Prof.S.V.Thorat, Dept. of MCA Yashoda Technical Campus,Satara-415003

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Abstract—

Computation that employees perform on their desktop and the management of the desktop computing infrastructure to the cloud, the need for securing such cloud-hosted user computing tasks and environments become paramount. In this paper, we present Venia, a secure cloud-based desktop computing platform designed to protect against both external and internal threats. Accessible to end-users through a thin Remote Desktop Protocol (RDP) client Venia isolates end-user's applications and data into containers and subjects the interactions with and among the containers to security policies. Following a principle of least privilege, Venia security policies control user's access to containers, network and file system interaction of the containers, cross-container data sharing and also enables collection of detailed logs for auditing purpose. Venia has been deployed to a 3rd party test environment where it demonstrated that end-users can perform the tasks they need on a daily basis, without introducing greater risk to the overall organization, and its currently undergoing security and performance evaluation by an independent evaluation team.

1. INTRODUCTION

The next step within the trend of moving backend services and supporting computing infrastructure to the cloud, is to maneuver end-user computing and its supporting infrastructure to the cloud additionally. Cloud computing provides economy of scale, eliminates the headache of computer code and hardware management and maintenance, and permits on-demand scaling and pay as you utilize rating. Properly architected, moving end-user computation to the cloud will offer a security profit. A conscientious cloud seller can offer stronger perimeter protection, specialised employees, and established tools, techniques and procedures for handling security incidents than a typical enterprise will generally deploy. However, sharing machine resources within the cloud presents a brand new set of security challenges for ensurinorganization and even worse, users from completely different organizations cannot breach security to attain malicious objectives.

2. Related Work

Secure Desktop computing in the cloud Current solutions for desktop computing within the cloud square measure based off of a Virtual Desktop Infrastructure (VDI) approach. VDI could be a variety of virtualization wherever entire desktop solutions are hosted within the cloud, so accessed employing a skinny consumer, usually with RDP. One such technology is Horizon seven by VMWare. in hand with these solutions is their wholesale exporting of the desktop atmosphere to the cloud. While helping to modify the digital geographic point and providing a centralized management over resource and network access, these solutions still maintain the appliance primarily based security problems inherent in a very ancient desktop.

3. Design Goals And Approach

The main style goals for Venia were:

• Role-specific UCEs: UCEs for individual users ought to consist of role-specific application bundles, wherever a job defines that desktop applications and resources area unit required to perform a particular job connected operate. A single user might have multiple roles, presumably requiring use of applications from multiple operative systems (e.g., Linux and Windows) in a very single role, and resources will be shared among completely different roles.

• Enterprise-specific security management and auditing:

Interaction between end-user skinny consumer and UCE ought to be encrypted. Resource access, data sharing and use of UCEs ought to be subject to enterprise-specific security controls and auditing policies.

• End-user expertise: End-user experience shouldn't change drastically from exploitation desktop atmosphere, in particular, end-users shouldn't need to re-authenticate themselves for role specific resource access, ought to realize familiar applications in their UCE, and be able to cut and paste wherever allowed by the enterprise policy.

• Administration: Venia ought to give a straightforward approach for administrators to outline enterprise-specific security and auditing policies, and a straightforward to outline role-specific application bundles and instantiating user-specific UCEs. To attain these goals, Venia was designed as distinct components a collection of microservices establishing the required enterprise IT services for a useful corporate infrastructure, a User cipher atmosphere (UCE) that defines the end-user space, together with their desktop, keep files and applications.

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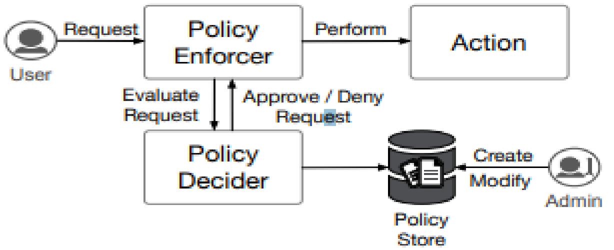

A. Enterprise IT Services as Microservices Enterprise IT services play a necessary and vital half in a corporate infrastructure. These services area unit liable for, among others, managing user access and authorization, and managing shared resources, like email servers, printers, and centralized filesystems. Venia contains a collection of microservices for performing aspects of those IT management functions, independent of the end-users space. Separating individual aspects of IT management responsibilities into distinct microservices that interoperate via a well-defined Representational State Transfer (REST) Application Programming Interface (API), and subjecting these interactions to strict security controls and auditing [6] reduces the chance of abusing the UCEs through the enterprise IT services, resulting in associate degree overall reduction of the attack surface of the UCEs.

Venia contains four microservices:

• **User Service:** provides the initial entry purpose into the Venia system, via a web-portal, and contains all of the business logic for authenticating a user against a directory service, like Active Directory, and obtaining all of their out there roles.

• **Virtue Service:** The Virtue Service coordinates communications between the opposite microservices, and is responsible for constructing the UCE. Once created, the only reference the UCE maintains back to the microservices is for coverage work events. This eliminates the potential for lateral attacks on the enterprise assets.

• **sensing element Service:** The sensing element service aggregates all of the logs across the Venia system. This centralized service provides the required observance and analysis of system activities.

• **Admin Service:** The admin service provides for the definition, management, and dissemination of policies

B. User Compute Environment (UCE)

UCE supports the acquainted daily interaction of the end-user to perform their daily tasks. The Venia UCE may be a single cloud based mostly machine instance that uses policy controlled containers to protect every Virtue, providing application isolation, and the ability to tightly management and monitor all actions and interactions. The UCE incorporates security mechanisms at multiple levels to ensure Associate in Nursing operational end-user expertise, whereas maintaining the goals and objectives of the policy.

4. Implementation

The current version of Venia is enforced as Associate in Nursing Amazon Web Services (AWS) application. This implementation consisted of two-subnets running in a very single Virtual Private Network (VPC). The sub-nets were divided between enterprise microservices in one, and UCEs in another. The only microservices that area unit accessible outside of the VPC area unit the Admin service, for policy construction, and also the Login service, for UCE creation.

A. UCE Implementation

Each Virtue lives through one LXC The display of every Virtue is shared to the host's X Server show to give a unified desktop look. The displays of every Virtue are shown within the sort of another window that identifies the containing Virtue. The Windows instance is connected throughRDP inside every Virtue on Associate in Nursing application basis. this permits Windows applications to own native support with the appearance of being on one seamless desktop among the Virtue. UNIX system applications area unit supported through the LXC containers the Virtues live to tell the tale. A writing board manager at the host level has been other to manage copy-paste options between the Virtue windows.

B. Demonstrative Examples

To verify our policy approach, we created and tested a few unique Virtues to exercise the capabilities of the system. Each of these Virtues were defined to address a specific security, or operational scenario.

5. Evaluation

To evaluate VENIA, we have a tendency to performed a series of performance overhead tests to estimate user perceptible overhead. For these many typical user operations, and compared against a regular desktop environment. every take a look at was conducted thrice, and the average was computed For these measurements, the quality desktop system was a VM on physically native hardware with four processor cores and 8GB of memory. VENIA was running on AWS t2.xlarge with four processors and 16GB of memory. To verify our policy approach, we have a tendency to created and tested many unique Virtues to exercise the capabilities of the system. Each of these Virtues were outlined to handle a particular security, or operational state of affairs. The automobile industry is investing in autonomous vehicles for driverless cars, which will have to analyze and make decisions on data that pertains to their surroundings for movements and directions. These vehicles need to transmit Data to the manufacturers so that they can track their usage and also get the required maintenance

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alerts. The data will be transmitted through networks resulting in congestion. To achieve low latency when accessing the network, it is necessary for the manufacturers to device new effective computing ways

6. CONCLUSION

As more front-end applications and computation continue to migrate to the cloud, the need for a secure and usable platform is paramount. With Venia, we have demonstrated an architecture for a secure cloud-based end-user computing solution. With this architecture, we were successful in separating enterprise IT functions from end-user tasks, which helped to reduce the amount of information available to an attacker while still providing an operable environment for the user. We further emonstrated that enterprise specific security controls and auditing requirements can be enforced on the UCEs, and provided an easy to use administrative toll to construct well defined policies for Virtues. Initial results show that running the applications in virtues within cloud-based UCEs subject to the applicable security controls and auditing policies do not drastically change the user's perception of the applications' response time, or constrain access to and use of information and resources they need to perform their job functions.

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DIRECTOR
Yashoda Technical Campus
Satara

Green Computing for Internet of Things

Harshal Gajanan Patil[1], Rasika Vishnu Tapase[2], Asst.prof.P.S.Gade[3], Asst.prof.V.V.Kadam[4]

[1st]Harshal Gajanan Patil,MCA YTC, Satara
[2nd]Rasika Vishnu Tapase, MCA YTC Satara
[3rd] Prof.P.S.Gade,[4th] Prof.V.V.Kadam Dept. of MCA Yashoda Technical Campus,Satara-415003

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Abstract— Cloud computing services are used to meet the ever-growing demand of IoT. Data centers are becoming one of the largest energy consumers to provide the infrastructure for the IoT paradigm. The demand for energy will increase in the future as more innovation emerges and technology follows new practices that lead to the adoption of green computing. Green computing strategies reduce the energy consumption of IoT devices without compromising performance. This white paper evaluates many aspects of green computing for IoT computing and analyzes key concepts, challenges, and mitigations.

Index Terms – Internet of Things (IoT), Cloud Computing, Edge Computing, Energy Consumption.

1. INTRODUCTION

The Internet of Things (IoT) brings together intelligent objects integrated into heterogeneous networks to monitor processes and make decisions. This is large-scale sensor data that is leveraged using computational resources. Green computing can use resources or do otherwise in an environmentally friendly way. It involves the development and removal of various elements used in computers to reduce the environmental impact. Companies are starting to invest in computing equipment made from recyclable materials. The purpose of green computing is to use computing resources and economically viable ways in an environmentally friendly manner. Figure 1 shows IoT device statistics by year. IoT devices are connected to various networks and their growth continues to accelerate as businesses embark on digital transformation. It also influences the spending and revenue of his IoT market in the world. These added devices also pose network security issues that need to be addressed accordingly.

2. BACKGROUND

Green computing

Green computing is the design and use of resources that are environmentally friendly and sustain computing power without degrading it. Resources used in computers are recycled after use. Companies making these devices should use less energy and be more biodegradable. The majority of IoT devices are energy efficient sensors, which has led to their massive use by industrial players. These sensors also help advance IT to use wireless networks efficiently. Data centers provide data storage and processing capabilities for big data. Cloud computing platforms face the challenge of increasing numbers of IoT devices. These IoT devices require low latency and mobility, which is why they employ edge computing for real-time services. Fog computing is a distributed computing paradigm aimed at connecting network devices at different computing layers. It provides IoT devices with low-latency responses that centralized cloud computing architectures cannot provide. Green computing focuses on preserving computing power while reducing energy consumption and being environmentally friendly. Computer CPU manufacturing technology has advanced, making it more energy efficient with each generation. However, as the number of computing devices in use has increased, it has become imperative to meet the demands of green computing. Green computing has been introduced to cloud computing to reduce energy consumption and reduce the use of harmful substances within devices.

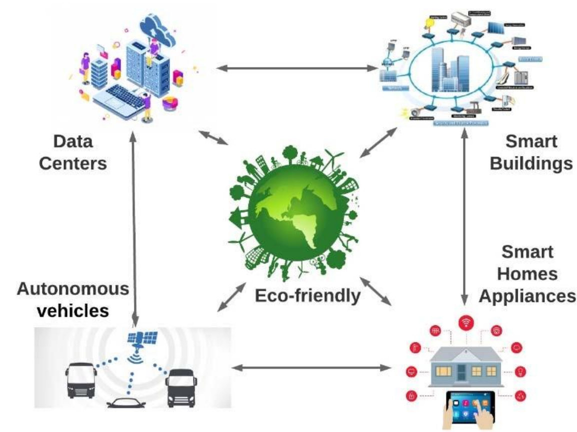

Figure 2: IoT green computing

Internet of things

IoT is the connection of devices to form an intelligent world. This is a paradigm that affects both society and technology. IoT technology involves building an infrastructure for connecting smart objects based on evolving information and network services. The data collected from the device must be processed for analysis and data protection regulations must be guaranteed. IoT is energy efficient when building smart cities. This is because the number of sensor devices and cooperating add-ons makes it easy for them to communicate with each other. Green computing must focus on reducing

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energy consumption to meet the sustainability and environmental friendliness of smart cities.

IoT has become a key factor in today's world, connecting devices critical to decision making. Most of these devices are sensors and devices that facilitate data exchange across various networks and enable information exchange between devices. This has led to the emergence of edge computing, enabling low-latency responses and reducing resource congestion in centralized data centers. Send all device data computations to a nearby edge data center. A distributed infrastructure also reduces network congestion that occurs during data transmission.

IoT applications rely on the internet for communication, manufacturing embedded hardware and managing IoT devices, and cloud computing for storage and processing. Finally, there is the presentation layer for interpreting the data that make up the various application layers of IoT, as shown in Figure 3. The architectural layer of IoT includes a perception layer made up of sensors and actuators. The network layer enables interconnection and communication between devices and transfers data. The application layer includes tasks such as displaying processed data and other abstract services. Figure 5 shows the different layers and their components classified according to their energy consumption.

Cloud computing

Cloud computing has resulted in the emission of CO2 due to the energy consumption from the data centers. Various practices have been adopted to lower the energy consumption by data center machines by using hardware virtualization and energy-Conversant strap in software applications. The energy consumption is predicted to rise with the continuous usage of cloud computing services and the data centers which host them. It is for this energy concern that there is a need to rethink how data centers adopt green computing, and the equipment been used [6]. An overview of IoT data been collected from devices, processed, and analyzed.

Edge-IoT

An increase in the rise of mobile devices has resulted in mobile edge computing (MEC) for low latency responses. MEC provides mobile computing, network congestion control, and storage capacity to the edges of the networks. MEC lowers the usage of mobile energy and supports latency-critical applications. The development of the 5G network has been motivated by the gains of MEC, which combines both wireless communications and mobile computing to offload network computation. Wireless sensor networks are responsible for sending data by indoor devices, at the front end of Wireless Mesh Sensor Networks (WMNs), edge devices are deployed to reduce the network congestion helping users to tailor their needs through MEC.

3. APPLICATION OF GREEN COMPUTING IN IOT

There are many government regulations to promote green computing. IoT edge computing has developed and benefited many industries. The various technological domains using green computing are:

Autonomous vehicles

The automobile industry is investing in autonomous vehicles for driverless cars, which will have to analyze and make decisions on data that pertains to their surroundings for movements and directions. These vehicles need to transmit Data to the manufacturers so that they can track their usage and also get the required maintenance alerts. The data will be transmitted through networks resulting in congestion. To achieve low latency when accessing the network, it is necessary for the manufacturers to device new effective computing ways

Edge computing aides the autonomous vehicles in transmitting and sharing the data between them. Edge data centers that are located at nearby geographical proximity helps in making the flow of data seamless. They also enable less usage of energy for sensors used in these autonomous vehicles. Since there is a shift towards the adoption of autonomous vehicles, the risk of carbon emissions is reduced, which will be a step forward towards the eco-friendly approach.

Smart cities

The data collected from sensors, which includes traffic, infrastructure, and home appliances, are used by city leadership to address the challenges witnessed in these cities. The data collected from these sensors are massive and requires extensive computing capabilities to process and analyze them; also, the response back to these devices should be in real-time, resulting in less usage of energy.

Industries

Industries such as oil drilling, can utilize IoT edge computing to gather data on a variety of environmental factors without relying on pre-collected historical data. Thus by adopting edge computing in industries, there will be lesser energy consumption in production .

4. ADVANTAGES OF GREEN COMPUTING

Green computing brings various benefits; some of them are:

Eco-friendly

Green computing reduces the negative impact of the manufacturing and disposal of computing devices in a manner that is eco-friendly and ensures environmental sustainability.

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Resource utilization

The data centers use resources such as computers for processing the collected data, the equipment used to make computer components should be biodegradable and also do not degrade on performance.

Low latency and Cost saving

Edge computing enables the allocation of resources in a manner that is efficient in energy consumption and reduces response latency. It also increases the lifetime of the devices saving on cost .

Improving on compliance

Green computing also enhances the compliance and regulation of the companies in meeting the business demands set by their customers and other stakeholders, also improving their image.

5. CHALLENGES FACING GREEN COMPUTING IMPLEMENTATION

Green computing awareness

People lack knowledge about green computing and its implications. According to the survey, he is only 28% aware of CO2 emissions and their impact on the environment.

Equipment cost

Businesses will have to pay to adopt green computing. People believe it in the traditional way. Savings over using modern energy efficient means. But these days, companies are controlling emissions by considering the energy consumption and carbon footprint of hardware devices.

Performance degradation

There are concerns regarding the materials used for making eco-friendly equipment resulting in performance degradation. Hence it is required to educate people regarding the usage of biodegradable devices and their performance.

6. SOLUTIONS TO THE CHALLENGES

Data centers

Data centers are an integral part of today's cloud computing industry. The energy consumption of these data centers should be reviewed regularly and steps taken to use biodegradable hardware components.

Virtualization

Data centers are an integral part of today's cloud computing industry. The energy consumption of these data centers should be reviewed regularly and steps taken to use biodegradable hardware components.

Recycling equipment

People discard unwanted hardware, which should be biodegradable so that it is eco-friendly. A lot of computer parts could cause harm to the environment. Thus, by using recyclable material, we can reduce the impact of these materials on the environment.

7. RESEARCH IMPLICATION

This white paper provided comprehensive insights on green computing for the Internet of Things. Green computing must adopt methods that reduce energy consumption and do not affect device performance. Companies should focus on energy efficient design of IoT devices and raise public awareness. Advancing green practices requires policy change and cooperation between organizations. This white paper presents challenges and possible solutions for green computing. Researchers can provide insight into best practices to follow to integrate green computing into the IoT. Green computing not only benefits the IoT but also promotes a cleaner environment.

8. CONCLUSION

IoT green computing is a top consideration when building a sustainable ecosystem. By adopting green computing practices, we can manufacture recyclable equipment and reduce energy consumption across our computing infrastructure. Green computing is therefore a great solution to support the growth of green IoT.

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Dairy Farm

Pratiksha Mahadik[1], Manasi Bhandari[2], Snehal Jadhav[3]
[1, 2, 3]*Computer Application, Shivaji University*

Abstract: A dairy farmhouse business is not like any other business. It takes a lot of hard work to run this business properly. So if you are thinking of starting this business then you must read our article. In today'sarticle, we are going to tell you the things that you need to take care of while starting this business. How can you run this business properly? But first of all, you need to know what is the status of this business in your country and how much profit you can earn from it. If you want to start a small business, you can starts a dairy business with government assistance.
Today we are going to give you information about dairy which is also known as a home business but is leading in making a profit Dairy farm business is a simple business but if you want to start a dairy business it requires more effort. In this article, you will get complete information about it, what is needed to start this business and how to make a profit.

I. INTRODUCTION

Dairy farming can be started by rearing cattle. Milk and milk products are used in almost all households. In the dairy farming business, you can earn money by rearing animals like buffalo, and cows and getting milk from them. Similarly, products such as cheese, curd, ghee, butter, sweets, etc. can be made and sold from milk. All these dishes take more time to prepare. And these can be sold at a higher rate than the cost of milk. Apart from this animal dung can be used in cow dung, and dung can be used as fertilizer on agricultural land. Beneficiaries can also earn money by selling fertilizer. Thus the process of doing business through animal husbandry is called dairy farming.

In this milk production business, we keep animals and get milk from these animals and distribute it in the market or nearby villages. If you have a large-scale dairy business, you can approach milk companies and supply milk to them, which earns a good income.

While opening a good dairy farm, we must have the following information- Cattle Information Cattle Information:

The most important point while opening a dairy farm is the choice of milking animals like cow, and buffalo, so while opening a dairy farm we should choose the cow, and buffalo properly, what breed of cow, and buffalo, what is its physical condition, and how much milk does it give? Because dairy farm depends only on buffalo and cow.

Complete information on Cattle Diseases: If you want to open a dairy farm, you must know about the main diseases of cows and buffaloes. Because if you don't know about the diseases of cow, buffalo, you don't know about many deadly diseases of your cow, buffalo and she will give less milk, eventually she will die and you will suffer huge loss.

II. METHODS AND MATERIALS

Entrepreneurs have to take care of certain things to start a dairy farming business. All those things arelisted below in the article:

1) First of all choose the location for your dairy business where you want to open a dairy business.
2) Then you have to choose which breed of cow, buffalo you want to keep.
3) There are many breeds of cows and buffaloes, of which the most milky breeds have to be kept.
4) Entrepreneurs can keep most popular and milch buffalo breeds – Mehsana, Murrah etc.
5) And popular breeds of cows are Jersey, Sahiwal, Friesian
6) For this you also need information about different castes.
7) All castes have different prices, candidates can choose the caste according to their budget

You can start dairy business in 3 ways:-

a) Small scale dairy farming
b) Moderate dairy farming
c) Large scale dairy business in India

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III. SMALL SCALE DAIRYING

To start a small scale dairy business you need less buffaloes and cows, in this you have to make a small budget and follow the same budget and move forward, in small scale you first take 2 buffaloes and 3 cows.When buying it, you want to make sure it is genuine (jersey). And because their milk will be sold at their breed rate you can also ask about fat wherever you sell milk. If you are doing 15-20 liter milk business then by selling that 20 liter milk you can earn up to 50 thousand per month and your investment in this will be up to 1.5 lakh once.

IV. MEDIUM SCALE DAIRY BUSINESS

Dairy farm is medium level average business, in this business you need 20 to 25 buffaloes and cows, in this also you have to make average budget and follow the same budget and make sure before buying cows buffaloes.

They are not true breed because their milk will be sold according to their breed rate (what class cow or buffalo belongs to i.e. if they are good class then their milk will be of good quality) because where you sell milk you can ask about fat if you drink 40 to 50 liters in a day. If you trade in milk you will make up to 1 lakh per month and your investment in this will be up to 2 lakhs only once.

V. LARGE SCALE DAIRYING

To open a large scale dairy farm you need specific types of buffaloes and cows, in this business you can start a business selling buffaloes or cows along with milk. But you also have to provide good food and fodder for them to eat. Because if you are going to open a large scale dairy farm, then your investment may also be high, the cost of good quality cows and buffaloes will also be high, so you will need 40 to 50 cows and buffaloes. On a large scale and you trade 300 to 350 liters of milk a day and you can save up to 10 thousand in a day, then you can earn up to 3 lakhs in a month and your investment in this is a one-time investment, it will not be repeated. Because you want to buy good food and fodder which ranges from 10 to 15 lakhs. And in addition to this, you can make and sell milk products such as paneer, khawa, cheese, buttermilk, butter, butter, ghee, kharwas, and some sweets. In this you will earn more than lakhs per monthand you will definitely benefit a lot in this business.

VI. HOW TO CHOOSE COWS AND BUFFALOES FOR DAIRY FARM

As we told you that if you want to be successful in dairy farm business, your first step should be what kindof cows and buffaloes you have on your farm. Because this is the main foundation of this business. There are different types of breeds of cows and buffaloes and their quality is also determined by the same breed, below we have listed the breeds of cows and buffaloes with grades. Which is as follows.

1) Mura – 20 liters to 25 liters of milk
2) Bhadavari – 16 liters to 20 liters of milk
3) Jafrabadi – 10 liters to 12 liters of milk
4) Surti – 8 liters to 10 liters of milk
5) Mehsana – 5 liters to 10 liters of milk
6) Nagpuri – 5 liters to 10 liters of milk

VII. ADVANTAGES OF DAIRY FARMING

1) Entrepreneurs can start their business from Ghadabishana.
2) This business has no impact on the environment.
3) Cow dung can be used for biogas.
4) It doesn't take a lot of people to start this business.
5) Dairying is a year-round business.
6) Manure can also be used in agriculture.
7) Farmers do this business to the maximum extent as it benefits them for crop along with dairy farming.
8) Banks also provide loans to start dairy farming.
9) Many schemes related to dairy farming are also being launched by the government.

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10) You can make good profit by producing milk and selling it in the market.
11) You can earn money by selling sweets or dairy products.
12) You can also earn profit from the milk products of cows and buffaloes

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Wi-Fi Technology

Shivani Ghadage[1], Swapnali Joshi[2], Shweta Thorat[3]
[1, 2]MCA Department, Shivaji University, Kolhapur
[3]Asst. Prof. MCA Department, Yashoda Technical Campus, Satara

Abstract: Technology is making rapid progress and is making many things easier. As the innovative thinking of persons is increasing day-by-day, new methods for wireless networking has been evolved of which our Wi-Fi, (Wireless Fidelity) is a trademark that belongs to the Wi-Fi Alliance. It is name commonly used for the standard of wireless (radio) connection that integrates several protocols and is based on a family of IEEE 802.11 standards (Institute of Electrical and Electronic Engineers is an international organization dedicated to the development of standards in electronic technology). The most famous and the most common today is IEEE 802.11g protocol, which determines the operation of wireless networks. Install Wireless LAN was recommended where the deployment of cable system was Wi-Fi, an acronym for Wireless-Fidelity which is the wireless way to handle networking. The main aim of this paper is wireless networking achieved by Wi-Fi. This paper introduces Wi-Fi technology and states the history of this technology in brief. We then deal with the different ways of wireless networking, connecting wi-fi and with wi-fi security. This paper concludes with the pros and cons of this technology and it's future.
Keywords: LAN, Wi-Fi technology.

I. INTRODUCTION

Impossible or economically impractical. Today, many organizations use Wi-Fi, since under certain conditions, the speed of networks now exceeds 100 Mbit / s. Users can still be connected to a Wi-Fi network, while moving between wireless access points (WAP). Mobile devices (PDAs, smart phones, PSP, laptops), equipped with Wi-Fi client transceiver devices, can be connected to the local network and access the Internet through a WAP or hotspot. Small bandwidth, no roaming and authentication capabilities do not allow Wi- Fi devices to press on the mobile market. However, such company as ZyXEL, SocketIP, and iSymbol Technologies offers solutions for Wi-Fi telephony.The history of Wi-Fi starts from mid-1990s. The technology of information transmission by radio has been developed and applied mainly in local networks of large corporations and firms in Silicon Valley, USA. Contact with a mobile subscriber (usually it was a company employee with a laptop equipped with a wireless network adapter) was organized through WAPs that were connected to a wired infrastructure. Thus, in the reach radius of each such point (a few tens of meters) could be up to 20 subscribers using the network resources simultaneously. Originally, the term «Wi-Fi» only used to refer to technology, which provides communication in the 2.4 GHz and runs on the IEEE 802.11b standard (baud rate– up to 11 Mbit / s). Currently, however, the term is increasingly used for other wireless LAN technologies. The most significant among them are the IEEE 802.11a and 802.11g (speed is up to 54 Mbit / s, frequency ranges, respectively, 5 GHz and 2.4 GHz).

Students, preparing their research proposal on the Wi-Fi, should use free example research topics on wifi security, which allow them to understand that 802.11b standard was developed in the late 90s and finally approved in early 1999. In 2000, there began to appear the first devices to transfer data based on it. Wi-Fi devices were intended for corporate users to replace traditional cable networks. For wired networks need careful design of network topology and manually laying many hundreds of meters of cable, sometimes in unexpected places. To organize the same wireless network, you only need to install one or more office base stations (central transceiver with an antenna connected to the external network or server) and insert a network adapter with antenna into every computer. Then computers can be moved arbitrarily, and even moving to a new office becomes possible keeping once created network.

Wi-Fi provides services in private homes, businesses, as well as in public spaces. Wi- Fi hotspots may be set up either free-of-charge or commercially, often using a captive portal webpage for access. Organizations, enthusiasts, authorities and businesses, such as airports, hotels, and restaurants, often provide free or paid-use hotspots to attract customers, to provide services to promote business in selected areas. Routers often incorporate a digital subscriber line modem or a cable modem and a Wi-Fi access point, are frequently set up in homes and other buildings, to provide Internet access and internetworking for the structure. Similarly, battery-powered routers may include a cellular Internet radio modem and a Wi-Fi access point. When subscribed to a cellular data carrier, they allow nearby Wi- Fi stations to access the Internet over 2G, 3G, or 4G networks using the tethering technique An Extended Service Set may be formed by deploying multiple access points that are configured with the same SSID and security settings.

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Wi-Fi client devices typically connect to the access point that can provide the strongest signal within that service set.[83] Increasing the number of Wi-Fi access points for a network provides redundancy, better range, support for fast roaming, and increased overall network-capacity by using more channels or by defining smaller cells. Except for the smallest implementations (such as home or small office networks), Wi-Fi implementations have moved toward "thin" access points, with more of the network intelligence housed in a centralized network appliance, relegating individual access points to the role of "dumb" transceivers. Outdoor applications may use mesh topologies..

II. WIRELESS NETWORK: WI-FI WALKIE- TALKIE NETWORK

To understand the wireless technology let us consider a pair of Walkie- Talkies.These are small radios that can transmit and receive radio signals. When we talk into a Walkie-Talkie, our voice is picked up by a microphone, encoded onto a radio frequency and transmitted with the antenna. Another Walkie-Talkie can receive the transmission with its antenna, decode our voice from the radio signal and drive a speaker. Simple Walkie-Talkies like this transmit at a signal strength of about 0.25 watts, and they can transmit about 500 to 1,000 feet. If we want to connect two computers together in a network using Walkie-Talkie technology:

1) Equip each computer with a Walkie-Talkie.
2) Give each computer a way to set whether it wants to transmit or receive.
3) Give the computer a way to turn its binary 1s and 0s into two different beeps that the walkie-talkie could transmit and receive and convert back and forth between beeps and 1s/0s.

This would actually work. The only problem would be that the data rate would be very slow. Walkie-talkie is designed to handle the human voice. So it is not being able to send very much data in this way(may be 1,000 bits per second).on any of three bands, or they can split the available radio bandwidth into dozens of channels Any products tested and rapidly between approved as "WiFi Certified" by the WiFi Alliance are certified as interoperable with each other, even if they are from different manufacturers. A user with a "Wi-Fi Certified" product can use any brand of access point with any other brand of client hardware that also is certified. Typically, however, any Wi-Fi product using the same radio frequency (for example, 2.4GHz for 802.11b or 11g, 5GHz for 802.11a) will work with any other, even if not "Wi-Fi Certified."

III. WI-FI RADIO TECHNOLOGY

The radios used in Wi-Fi are not so different from the radios used in walkie- talkies. There are three big differences between Wi-Fi radios and Walkie-talkies.

1) WiFi radios that work with the 802.11b and 802.11g standards transmit at 2.4 GHz, while those that comply with the 802.11a standard transmit at 5 GHz. Normal walkie- talkies normally operate at 49 MHz. The higher frequency allows higher data rates.
2) WiFi radios use much more efficient coding techniques that also contribute to the much higher data rates. For 802.11a and 802.11g, the technique is known as orthogonal frequency-division multiplexing (OFDM). For 802.11b, it is called Complementary Code Keying (CCK).
3) The radios used for WiFi have the ability to change frequencies. 802.11b cards can transmit directly them. The advantage of frequency hopping is that it is much more immune to interference and can allow dozens of WiFi cards to talk simultaneously without interfering with each other.

IV. CONCLUSIONS

As Wi-Fi is now shipped in millions of products and deployed in millions of homes, business and hotspots worldwide, the technology has moved beyond the realm of a computer feature. Wi-Fi has fast become a cultural phenomenon.

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RESEARCH ON ESTERIFICATION REACTION UNDER, MICROWAVE ASSISTED SYNTHESIS OF BUTYL BENZOATE FOR GREEN CHEMISTRY

Aishwarya A. Jadhav, Ms. Rasika Purushottam Devale, Mr. Kaustubh Chandrakant Jagtap
Ms. Swapnali Dhananjay Patil

Department of B Pharmacy
Yashoda Technical Campus, Satara.

Abstract: **In this research we explain all the detailed information about, Microwave assisted synthesis of Butyl Benzoate for esterification reaction in working green chemistry esterification reaction under Microwave assisted synthesis very much beneficial into assisted synthesis chemical reaction for green chemistry. Work by synthesis of butyl benzoate. This is initially used by the save energy and rate of reaction is fast. On this research work esterification reaction process of combining an organic acid (RCOOH) with and alcohol (ROH) to form Ester (RCOOR) and water. Some alcoholic groups are changes than chemical reaction resulting in formation least product butyl benzoate is ester obtained by esterification reaction, so alcoholic group are using n-Butanol and carboxylic group benzoic acid in the presence of conc. Sulfuric acid so, finally product butyl benzoate is forming an ester this synthesis microwave capable of predicting many properties and role of synthesis reaction is fast in small period of time to get form product. All type esterification chemical reaction synthesis is also done by microwave. Analyzed product by studying Thin Layer Chromatography. Various authors work on their subject by using this microwave assisted synthesis I show interest into microwave because of this is very beneficial for performing synthesis of butyl benzoate.**

Keywords: **esterification reaction Microwave assisted synthesis, green chemistry, Microwave, synthesis of butyl benzoate, Thin Layer Chromatography.**

INTRODUCTION 1

Green chemistry is defined as environmentally benign chemical synthesis of esterification reaction Microwave assisted synthesis in that microwave is a general-purpose green chemistry for performing organic synthesis reaction in small period of time and no purification necessary as compared to conventional heating method.[1] Microwave initially started used in or released in 1986 by the groups of Gedye and Giguere/ Majetich although the use is microwave heating in chemical purpose can be back to 1950. Esterification reaction Microwave assisted synthesis if focusses on a process whether carried out in industry or chemical laboratory. The reduced the use and generation of harmful substance or byproduct. On this research work esterification reaction process of combining an organic acid (RCOOH) with and alcohol (ROH) to form Ester (RCOOR) and water. Some alcoholic groups are changes than chemical reaction resulting in formation least product butyl benzoate is ester obtained by esterification reaction, so alcoholic group are using n-Butanol and carboxylic group benzoic acid in the presence of conc. Sulfuric acid so, finally product butyl benzoate is forming an ester this synthesis microwave capable of predicting many properties and role of synthesis reaction is fast in small period of time to get form product. [1][2] Analyzed product (Butyl benzoate) by studying method: Thin Layer Chromatography. Microwave assisted synthesis is the benefits of microwave esterification reaction process of combining an organic acid (RCOOH) with and alcohol (ROH) to form Ester (RCOOR) and water. Some alcoholic groups are changes than chemical reaction resulting in formation least product butyl benzoate is some reagent or reaction are benefits of microwave assisted synthesis [3]

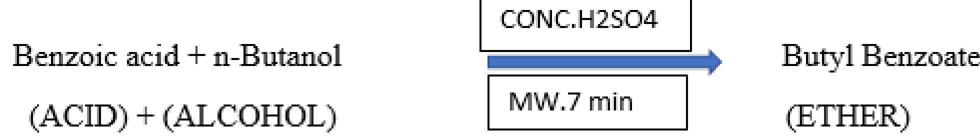
Fig. structure of Butyl benzoate

Butyl benzoate is a benzoate ester obtained by condensation of benzoic acid and butanol. It is used as a perfume ingredient and as a solvent for cellulose ether, a dye carrier for textiles. It has a role as an antimicrobial food preservative, a fragrance and a plant metabolite.[3]

1) Faster reaction synthesis of esterification reaction esterification reaction in microwave 7 min.
2) Better yield and higher purity (microwave synthesis of Butyl benzoate).
3) Energy saving for esterification reaction microwave synthesis of Butyl benzoate.
4) Uniform and selective heating.
5) Esterification reaction microwave synthesis of Butyl benzoate is green synthesis. [4–7]

PRINCIPLE OF ESTERIFICATION REACTION MICROWAVE SYNTHESIS OF BUTYL BENZOATE AS COMPARED TO CONVENTIONAL HEATING METHOD: 2

In microwave assisted synthesis and conventional heating method synthesis are same reaction but only heating method are deferent in microwave heating method are very beneficial for performing synthesis of butyl benzoate. Or other esterification reaction and all chemical reactions. And also rate of reaction is fast. By using dehydrating reagent Sulphuric acid (H2SO4). Also, Sulphuric acid acts as a catalyst in this reaction and removing water molecule. [9-12]



Some esterification reaction synthesis on microwave in general working on following way in short period of time and rate of reaction is fast.[13]

As compared to conventional heating method by synthesis of butyl benzoate in general working on round bottom flask add 3gm benzoic acid and add Butanol 5ml and CONC.H2SO4 are 2 to 3 drops. Then conventional heating 45min product are obtained. Same process working on microwave heating apply (electrical heating is converted to heat energy) product are obtained only 6min.very fast reaction in microwave as compared to conventional heating method by synthesis of butyl benzoate. [14-15]

Butyl Benzoate are compared by two heating method, microwave synthesis heating method and conventional synthesis heating method: 3

Sr.NO.	Heating Method	Green chemistry applies	Temperature 0C	Time	Purity after recrystallization	Stability 1gm in 1ml H2O
1.	Microwave	yes	60 W	6min	96%	Practically insoluble
2.	Conventional	No	60 0C	45min	84%	Practically insoluble

Table No.1

ANALYZED PRODUCT (MICROWAVE SYNTHESIS OF BUTYL BENZOATE) BY STUDYING THIN LAYER CHROMATOGRAPHY:4

Analyzed Product (Microwave Synthesis of Butyl Benzoate) By Studying Thin Layer Chromatography in that thin layer of silica gel spread on a glass surface than Butyl Benzoate spot the plate using a capillary tube.[16] Press the tube firmly to the plate in order to deposit the solution. We will use solvent to develop the plate. put solvent in a beaker and insert the plate. Mark sure the line sits above the solvent (Butyl Benzoate) and cover the beaker. than now we watch the plate develop.[16]

Mobile phase are water and methanol (1:2) ratio. And stationary phase by using silica applying spot Butyl Benzoate than calculate difference between Microwave Synthesis product and Conventional synthesis product are following:

Formula: RF Value = $\frac{\text{distance travelled by solute}}{\text{distance travelled by solvent}}$

i.e., d0 = travel distance of the solvent.
dA = travel distance of the compound A (microwave synthesis product).
dB = travel distance of the compound B (Conventional synthesis product).
R1 = (Ratio of front) value for A = dA /d0.
R2 = (Ratio of front) value for B = dB /d0.

Sr.NO.	Heating Method	travel distance of the solvent. (d0)	travel distance of the compound (d)	Ratio of front value (R)
1.	Microwave	6cm	(Spot A) 5.2cm	0.79
2.	Conventional	6cm	(Spot B) 4.7cm	0.71

Table No.2

Result: The Analyzed Product (Microwave Synthesis of Butyl Benzoate) By Studying Thin Layer Chromatography are less absorption in that less affinity, So Butyl Benzoate are movement fast. And as compared to conventional synthesis of Butyl Benzoate are more absorption in that more affinity, So Butyl Benzoate are movement are slow.[17]

REVIEW OF LITRETURE

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Microwave radiation, an electromagnetic radiation, which is widely used as a source of heating in organic synthesis Microwave-Assisted Synthesis: Review of Recent Developments Neha Gupta Department of Chemistry, Dev Samaj College for Women, Ferozepur City.18th-19th march 20017. [15]
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CONCLUSION: Esterification Reaction Microwave Synthesis of Butyl Benzoate as Compared to Conventional Heating Method to change the alcoholic group (butanol) product Microwave Synthesis of Butyl Benzoate are within 6min are Obtained synthesized product. And Conventional Heating Method synthesis product are within 45min are Butyl Benzoate are Obtained synthesized product.
To Analyzed Product (Microwave Synthesis of Butyl Benzoate) By Studying Thin Layer Chromatography and to check purity of Butyl Benzoate was found to be 0.79 As Compared to Conventional Heating Method by Studying Thin Layer Chromatography and to check purity of Butyl Benzoate was found to be 0.71.

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Review On General Purpose Of Catalysis In Green Chemistry

Saraswati.M.Pawar, Rasika.P.Devale

Department of B Pharmacy, Yashoda Technical Campus, Satara 415001

Abstract

Green chemistry, also known as sustainable chemistry, refers to the development of chemical products and processes that minimize or eliminate the usage and production of harmful compounds. They only utilize environmentally friendly chemicals and chemical procedures. It is built on twelve principles that can be used to develop or reproduce molecules, materials, reactions, and processes that are safer for human health and the environment from the ground up. Green Chemistry decreases the environmental impact of chemical processes and technologies, as demonstrated in this article.

The goal of this research is to learn more about the role of catalysts in green chemical synthesis for a more sustainable future. In the ecologically friendly synthesis of novel and existing compounds, catalysis plays a critical role. Catalyzed processes require less energy to produce and produce fewer by-products, co-products, and other waste items, indicating increased efficiency. Catalysts can be created in such a way that they are not harmful to the environment. Catalysts come in a variety of shapes and sizes, and some of them have positive effects in the chemical industry.

Key words- Biocatalysis, Biomass, Ionic Liquids, Critical Fluids, Microwave Irradiation, Photocatalysis, Green Chemistry

Definition of green chemistry-

Green chemistry, also known as sustainable chemistry, is the development of chemical products and processes that reduce or eliminate the usage and manufacture of harmful compounds.[1] Chemical goods should be designed so that they do not persist in the environment after they have served their purpose and are broken down into environmentally friendly components.[2]

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INTRODUCTION TO GREEN CHEMISTRY-

In the early 1990s, the concept of green chemistry was originally proposed. The first volume of the well-established green chemistry journal of the Royal Society of Chemistry was published in 1999, and the green chemistry institute was founded in 1997.[3] Green chemistry processes encompass practically all aspects of chemistry, including inorganic, organic, biochemistry, polymer, environmental, and toxicity. The goals of environmental protection and economic benefit can be achieved through several prevailing trends of the green programme, such as catalysis, bio-catalysis, and the use of safety alternatives: renewable feedstock (biomass), reaction solution (such as water, ionic Liquids, and supercritical liquids), reaction conditions (microwave irradiation), and new synthetic pathways (photo catalytic reaction).[4]

Concept of Pharmaceutical Green Chemistry

Pharmaceuticals are the most dynamic segment of the chemical business. It is at the vanguard of major shifts toward “greener” feedstock, cleaner solvents, alternative methods, and new concepts. All of these measures will improve the pharmaceutical industry’s environmental credentials while also lowering costs and materials for manufacturing processes, paving the way for long-term sustainability.Green chemistry is a Hippocratic oath for chemists, and a new generation of scientists and technologists is being formed to analyse the processes and materials used in production and development efficiently in order to protect natural resources and the environment. If no hazardous substances are used or produced, the risk is zero, and there is no need to be concerned about removing hazardous substances from the environment or limiting exposure to them. “Green chemistry is about reducing waste, raw materials, risks, energy, environmental impact, and cost,” as the phrase goes.[5]

Scientific Areas for Practical Applications of Green chemistry

The areas proposed for special focus under the green chemistry Principles were the following.

1.Use of alternative feedstock

2.Use of less hazardous reagent

3.Use natural processes like biocatalytic techniques

4.Use of alternative solvents

5.Designe of safer chemicals and products.

Green Chemistry's Latest Trends-

The green program’s core goals are achieved through many prominent trends in the design, development, and use of chemical products and processes that decrease or eliminate the use or production of substances that are dangerous to human health and the environment.”

a. Catalytic and biocatalytic reaction research in order to obtain highly selective, pure compounds without the formation of toxic byproducts;

b. Searching for new raw materials that are both harmless and renewable, such as biomass;

c. Developing environmentally friendly chemicals that are less toxic;

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d. Developing and evaluating new non-toxic, renewable reaction media, such as water, ionic liquids, and supercritical fluids.

e. Developing and evaluating new reaction conditions, such as microwave, ultrasound, and light reactions.[6]

PRINCIPAL IN GREEN CHEMISTRY

There are twelve green chemistry principles that have been created By EPA's Paul Anastas and John Warner, who described their significance in practise in their Green Chemistry Theory and Practice book, published in 1998. Green chemistry principles call for the elimination or reduction of dangerous or harmful compounds from the synthesis, manufacture, and application of chemical products, reducing or eliminating the use of substances harmful to human health and the environment.

"Reducing Risk" and "Minimizing the Environmental Footprint" are two of the principles. In the past, various chemical industries have been associated with risk. Hazardous chemicals to humans and the potential of environmental pollution were linked to new chemical products, giving synthetic chemical materials a "bad name." Energy use, climate change, crisis, and depletion of natural resources are all factors in the environmental footprint.[7]

1.Prevention

2.Atom Economy

3.Less Hazardous Chemical Syntheses

4.Designing Safer Chemicals

5.Safer Solvents and Auxiliaries

6.Design for Energy Efficient

7.Use of Renewable Feedstock

8.Reduce Derivatives

9.Catalysis

10.Design for Degradation

11.Real-time analysis for Pollution Prevention

12 Inherently Safer Chemistry for Accident Prevention

What is Catalysis-

Catalysis is a term used in chemistry to describe the process of modifying the rate of a reaction by using a substance that isn't consumed by the reaction.

How it related to green chemistry

Chemical operations produce large amounts of trash every day. Stoichiometric equivalents, in particular, produce undesirable byproducts such as inorganic salts. More efficient catalytic alternatives are progressively replacing stoichiometric chemical methods, allowing scientists to save energy and resources. Moving away from stoichiometric processes and toward homogeneous and heterogeneous catalytic reactions using organic, organometallic, inorganic, and biological catalysts is referred to as greener catalysis.[8]

Role of Catalyst in green chemistry

Green chemistry is an area of chemistry that focuses on the discovery and use of environmentally friendly chemicals and processes . Catalysis is a key component of green chemistry. Green chemistry, often known as environmentally benign chemistry or sustainable chemistry, minimises toxicity. Its objective is to design and execute pollution avoidance solutions other than waste management that reduce waste, save energy, and reduce natural resource depletion. Green chemistry is considered environmentally friendly because it is thought to reduce carbon emissions and pollution. Catalysis has aided in the reduction of pollution in our environment. Catalysts have been used to improve air quality by removing and controlling NOx emissions, reducing the use of Volatile Organic Compounds (VOCsi), developing alternative catalytic technology to replace the use of chlorine or chlorine-based intermediate in chemical synthesis and waste minimization, and developing alternative catalytic technology to replace the use of chlorine or chlorine-based intermediate in chemical synthesis and waste minimization. Catalysis allows for more efficient and selective reactions, resulting in the elimination of vast volumes of by-products: and other waste chemicals.[9]

Types of Catalysis –

Depending on the number of phases in which the catalytic reaction is carried out, homogeneous or heterogeneous catalysis can be used for synthetic processes.Homogeneous catalysis is a single-phase reaction that is usually liquid/liquid, whereas heterogeneous catalysis is a multi-phase reaction. The use of homogeneous catalysts provides a number of advantages, including decreasing reaction temperatures and thereby saving energy.[10]

The following are some of the catalysts:

1.METAL CATALYST – Using well selected metal catalysis can make a reaction more ecologically friendly. Transition metals are frequently utilized as catalysts in reducing reactions like hydrogenation.Metal catalysts can be pure metals or bimetallic or multimetallic mixtures of metals, or they can be spread on solid supports like silica, alumina, or carbon.[11]

2)METAL OXIDE CATALYST- For catalytic oxidation, transition metal oxides have been utilized. In the production of bulk chemicals, molecular oxygen is preferred, whereas in the production of fine chemicals, hydrogen peroxide is preferred. Although more expensive than molecular oxygen, hydrogen peroxide is environmentally friendly because it is converted to water during the oxidation reaction.Because it is transformed to molecular oxygen, ozone is also environmentally friendly, but its generation necessitates particular handling and equipment.[12]

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3)METAL COMPLEXES- In homogeneous catalysis, metal complexes are commonly utilised. A transition metal complex was used to synthesise naproxen with a 97% yield under high pressure. Chiral metal complexes catalyse inhomogeneous phase reactions while also controlling the reaction's stereo specificity.[13]

4)BIOCATALYSTS- Enzyme and antibody catalysts are used in both homogeneous and heterogeneous systems.

•ANTIBODY CATALYSTS- Another form of biocatalyst that is frequently employed is antibody catalysts. Antibody specificity and selectivity are related to the antigen structure required to elicit an immune response.

•ENZYME CATALYSTS**-** Selectivity is one of the most notable characteristics of enzyme catalysts. They are regioselective, which means they can distinguish between several identical groups within the same molecule. Enzyme catalysis can take place in both aqueous and non-aqueous solvents, including supercritical fluids.[14]

Solid acid and bases As catalyst-

Acid and base catalysed reactions are important in the oil refining and petrochemical sectors, as well as in the production of a wide range of speciality chemicals like medicines, agrochemicals, and flavors and perfumes. In liquid-phase homogeneous systems or on inorganic supports in vapour phase systems, many of these processes require the use of conventional Brnsted acids (H2SO4, HF, HCl, p-toluene-Sulfonic acid) or Lewis acids (AlCl3, ZnCl2, BF3). Similarly,NaOH, KOH, NaOMe, and KOBut are examples of common bases. As a result of their subsequent neutralisation,The formation of inorganic salts that eventually find their way into aqueous streams.[15]

Additional advantages of using solid acids and bases as catalysts include:

-Separation and recycling are made easier, resulting in a faster process and lower production costs.

-Solid acids, such as H2SO4, HF, are safer and easier to handle than their liquid equivalents. Very corrosive and necessitates the use of costly construction materials

-Trace levels of (neutralized) catalyst contamination in the product are often avoided.

When the latter is a dependable.

-Granular chemicals are safer and easier to operate than their liquid counterparts.

Solid Acid Catalysis –

One of the most important applications of heterogeneous catalysis is in acid-catalyzed processes. Solid catalysts are utilised in a wide range of applications. Acidic Clays, zeolites, supported heteropoly acids, and mixed oxides like silica–alumina and sulfated zirconia are among them.Hybrid organic–inorganic materials, such as mesoporous oxides, and organic ion exchange resins Organic sulfonic acid moieties are suspended in the air.[16]

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WITH A SOLID BASE

There are much fewer examples of reusable solid base catalysts in use than there are for solid acids.This is most likely due to the fact that acid-catalyzed reactions are far more common in the manufacture of Substances that are widely available. The different types of solid bases that have been reported are similar.Anionic clays, basic zeolites, and anionic clays are all alternatives to the solid acids detailed in the preceding sections.Mesoporous silica grafted with organic bases pendent.[17]

Catalytic C–C Bond Formation

Another important transformation in organic synthesis is the production of C–C bonds, and carbonylation is an important catalytic technique for producing C–C bonds. It's utilised in the bulk chemicals industry to make acetic acid by catalysing the carbonylation of methanol with rhodium and since they are 100 percent atom efficient, they are increasingly being used in fine chemistry.Manufacture of chemicals The Hoechst-Celanese method, for example, is a beautiful illustration of this.Manufacturing of the analgesic ibuprofen, with a production capacity of several thousands of tonnes per year.[18]

TECHNOLOGY OF ENZYMES IN BIOCATALYTIC REDUCTION

Reductions are important in organic synthesis because they lead to chiral compounds with new functionalities.Such processes can be catalysed by enzymes with exceptional stereo-, regio-, and chemoselectivity, resulting in The path to not just high-added-value but also shorter classical synthetic pathways Compounds, as well as bulk chemicals, are available.Enzymes, nature's catalysts, offer nearly limitless access to a wide range of chemical reactions.Reactions. Reductions in particular can result in the formation of not just multiple chiral centres, but also multiple chiral centres.But also new functional groups in products that are in high demand in the pharmaceutical and fine chemical industries.[19]

Are Biocatalytic Reactions Green?

Today, the statement "biocatalysis is intrinsically green" has become a mantra for many Researchers.First of all, researchers should be aware that no chemical transformation (including Biocatalytic reactions) is green, as in all cases resources are consumed and waste is generated, Thereby putting a burden on the environment. We believe that a given reaction of methodology can Be greener than another reaction. Such a comparison, however, should be based on quantitative Data rather than on general statements. Comparative full life cycle assessments (LCA) represent The "gold standard" for such comparisons, but are usually time-intensive due to the large data basis Required for a meaningful comparison. Sheldon's E-factor6 and possibly its derivative, the E+-Factor, taking energy-related CO2 emissions into account,7 represent an acceptable alternative for The preparative chemist.[20]

Conclusion

There is a need to update or adapt traditional procedures that are not environmentally friendly, use dangerous solvents, and are not atom specific in the sense that they do not follow green chemistry principles. This could be beneficial to students' safety while also being environmentally sustainable. For the first time, a new approach has been established. IN organic synthesis, non-conventional approaches are used. Catalysis is crucial in the environmentally friendly synthesis of compounds. By substituting an environmentally friendly synthetic approach for a standard synthetic pathway, several by-products, co-products, possible wastes, and pollutants can be avoided. The reduction of a number of steps that normally occur during synthesis shows the possibility for catalyst to be employed for environmentally friendly synthesis. The use of catalysts in chemical synthesis can be quite beneficial. Inventing environmentally friendly technology and producing ecologically friendly chemicals.

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RESEARCH ARTICLE

Influence of Newly Synthesized Superdisintegrant on Dissolution Rate Enhancement of Carbamazepine using Liquisolid Compact Technique

Vrushali G. Raut[1]*, Bharatee P. Chaudhari[2], Vivekkumar K. Redasani[3]
[1]M. Pharmacy Student, Department of Pharmaceutics, YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Satara 415011, (M.S.) India.
[2]Assistant Professor, Department of Pharmaceutics, YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Satara 415011, (M.S.) India.
[3]Principal, YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Satara 415011, (M.S.) India.
*Corresponding Author E-mail: **vgraut148@gmail.com**

ABSTRACT:
The purpose of this study was to manufacture liquisolid compact of high dose poorly water-insoluble drug, Carbamazepine (CBZ) by using novel superdisintegrant for the purpose of fast disintegration and improved its dissolution rate. The solubility of CBZ was analyzed in various non-volatile solvents in order to find the vehicle with the maximum solubility. The dissolving profile of liquisolid compacts was compared to a marketed tablet formulation's dissolution profile. CBZ was found to be much more soluble in polyethylene glycol 200 than in the other solvents. Crosspovidone-containing formulations showed no disintegration, but all other formulations disintegrated after 91.2 seconds. A Starch Glutamate-Croscarmellose Sodium combination has a disintegration time of 42.5 seconds. The optimized batch NSC1 including Starch Glutamate-Croscarmellose Sodium had 94.81 % greater drug release compared to the marketed formulation. This investigation found that the novel superdisintegrant had the fastest disintegration and the highest drug release compared to other disintegrants.

KEYWORDS: Liquisolid Compact, Fast Disintegration, Dissolution Enhancement, Starch Glutamate, Carbamazepine, Neusilin.

1. INTRODUCTION:

To be absorbed from the gastrointestinal tract, the oral solid dose form must dissolve. Water-insoluble drugs have poor dissolving rates and absorption characteristics, which are major concern for the pharmaceutical industry [1]. Liquisolid compacts have recently emerged as a potential approach for enhancing the dissolution rate of poorly soluble drugs[2]. The notion of "liquisolid systems" as defined by Spireas et al. i.e Simple physical blending with selected excipients termed the carrier and coating material can be utilised to transform a liquid into a free flowing, readily compressible, and apparently dry powder[3].

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The drug's wetting properties and surface area that is available for dissolution are considerably improved by the liquisolid compact. Water-insoluble compounds in liquisolid compacts are likely to have increased drug dissolution, resulting in improved bioavailability[4]. The liquisolid compact approach has been successfully used to improve the in vitro release of poorly soluble drugs such as indomethacin,[5] piroxicam,[6] griseoful¬vin,[7] ezetimibe,[8] repaglinide,[9] prednisolone,[1] etc. The liquisolid approach has been successfully used to improve the release of low dose, poorly soluble drugs. However, one of this technology's limitations is the conceptualization of a high-dose poorly soluble drug[10]. In order to enhance drug loading, the powder must retain high amount of liquid. However, this may result in poor flow and compression characteristics of the powder. A large amount of carrier and coating component should be used to maintain good flow and compression properties.

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As a result, increasing the capacity for liquid adsorption with carrier and coating component such as Neusilin could be a potential approach to loading a high dose of water insoluble drug. Neusilin US2 is an amorphous synthetic form of magnesium aluminometa silicate[11].

Carbamazepine (CBZ) has been used for over 40 years to treat epilepsy and trigeminal neuralgia. Carbamazepine is taken in doses of 100-200mg once or twice daily. It has a 72-96% oral bioavailability. It is practically insoluble in water. CBZ oral absorption in humans is slow, erratic, and unpredictable due to slow dissolution. One of the most major issues with this drug is its very low solubility in biological fluids, which results in poor bioavailability after oral administration. Many trials have been conducted in order to improve CBZ bioavailability. The use of water-soluble salts and polymorphic forms, the formation of water-soluble molecular complexes, Amorphisation of drug[12], Micronization[13], Solid dispersion[14], Co-grinding[15], Self-emulsifying drug delivery system[16], Nanosuspension[17], Hot melt extrusion[18], Adsorption of drugs to hydrophilic silica aerogels[19], Lyophilization, microencapsulation, and Inclusion Complexation[20,21] are some of the most important formulation tools. Apart from that, this method of liquisolid compact formulation is one of the method for increasing the rate of dissolution of poorly soluble drugs[22]. Superdisintegrants are the most common excipients used in tablet formulations to speed up disintegration in the gastrointestinal environment and thus increase active ingredient release. Disintegration is a critical step in drug release and absorption into the systemic circulation, resulting in pharmacological effects. However, the number of available superdisintegrants is still limited, necessitating the development of more efficient ones. The addition of Starch Glutamate, a hydrophilic amino acid, to the starch may improve its ability to disintegrate. The prepared formulation was characterised and compared to marked formulations (Mazetol)[23].

2. MATERIALS AND METHODS:

2.1. MATERIALS:

CBZ was received as gift sample from Abbott Healthcare Pvt. Ltd. Mumbai and chemicals were obtained from Loba Chem in Mumbai.

2.2. METHODS:

2.2.1. Synthesis of novel superdisintegrant:

To make a starch slurry, 10 parts potato starch were accurately weighed and dispersed in 25 parts distilled water. Weighing and dissolving 10 parts glutamic acid in distilled water, it was added to the starch slurry. The dispersion was conditioned for 16 hours after adjusting the pH to 3.5 with 10ml sodium hydroxide to complete the reaction between potato starch and glutamic acid. The dispersion was washed to remove unreacted glutamic acid after conditioning, and the solid mass was dried at 60°C to yield starch glutamate. To get consistent sized particles, the dried starch glutamate was sieved with a #120 sieve and kept in desiccators[24].

2.2.2. Characterization of novel superdisintegrant:

2.2.2.1. Fourier transformed infrared spectrometer (FTIR):

The molecular substitution of starch glutamate was evaluated using a Fourier Transform Infrared Spectrometer (FTIR). The IR spectrum of starch glutamate was measured using an ATR Fourier Transform Infrared Spectrophotometer (Shimadzu, Japan, IRAFFINITY-1Miracal 10). A small amount of sample was taken and directly put on the ATR diamond. The sample was pressurized using a pressure arm. The spectrum was then scanned in the wavelength range of 4000-400cm^{-1} [23].

2.2.3. Solubility studies:

Saturation solubility studies in four different non-volatile solvents, namely PG (propylene glycol), PEG 200, PEG 400, and Tween 20, were needed to identify the appropriate non-volatile solvent for making liquid medication. Excess amount of carbamazepine was mixed separately with four non-volatile solvents. For 48 hours, the mixtures were shaken on an orbital shaker using the shake flask method. The solutions were then filtered through Whatman filter paper to obtain clear solutions. These filtered solutions were often diluted with 1% SLS (sodium lauryl sulphate) and their drug content was determined using UV spectrophotometry at 285 nm. To calculate carbamazepine solubility, three determinations were performed for each sample[22].

2.2.4. Determination Value:

The flow properties of powder excipients (Neusilin US2) in liquid vehicles were assessed using the "angle of slide" measurement. Several homogeneous liquid vehicle/powder admixtures containing 10 g of carrier or coating ingredients and increasing volumes of liquid vehicle were prepared (PEG 200). The created powder admixtures were placed on polished metal plates, which were gradually tilted until the powder admixture was about to slide, to measure the angle of slide. The angle of slide (ø) was used to describe the angle formed between the plate and the horizontal surface. The flow properties of excipients will be altered due to adsorption of the liquid vehicle. The flowable liquid-retention potential (ɸ-value) of each liquid/powder admixture was evaluated using the following equation.

Φ value = liquid weight/solid weight

The graph were plotted against ɸ -values versus the corresponding angle of slide (ø). The flowable liquid retention potential, ɸ -value, of its powder, which is required for the preparation of liquisolid tablets, was

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represented by an angle of slide (for optimal flow properties) corresponding to 330 of a liquid/powder admixture. All measurements were taken in triplicate[25].

2.2.5. Liquisolid system preparation:

The amount of excipients is determined by their ɸ-values and liquid load factors. In the current research, neusilin was used as a carrier and coating material. The liquid load factor (Lf) is calculated using the formula below

Lf = ɸ + ø (1/R)……………………………………... (1)

Lf =W/Q …………………………………………….. (2)

R=Q/q ………………………………………………. (3)

Where, Φ and ø are the values of the carrier and the coating powders respectively, while R is excipient ratio[1].

In PEG 200, CBZ was suspended, as indicated in the table no. 3, and a total of 12 batches were formulated, as indicated in the Table No.1.

2.2.5.1. Preparation of CBZ liquisolid compact:

The carbamazepine, carrier and coating material, and other excipients in a liquisolid powder mixture were immediately compacted on a single punch tablet machine to yield tablets with the specified diameter, thickness, and hardness[22].

Table No.1 Formulation of Liquisolid Compact of Carbamazepine

Name of superdisintegrant	Batch code	%CD	LF (mg)	R (mg)	W (mg)	Q (mg)	q (mg)	Superdisintegrnat (mg) 3%,5%, 7%	Total Weight (mg)
Crospovidone	NCP1	50%	1	20	200	200	10	3%-15.3	525.3mg
	NCP2	50%	1	20	200	200	10	5%-25.5	535.5mg
	NCP3	50%	1	20	200	200	10	7%-35.7	545.7mg
Crosscarmallose Sodium	NCS1	50%	1	20	200	200	10	3%-15.3	525.3mg
	NCS2	50%	1	20	200	200	10	5%-25.5	535.5mg
	NCS3	50%	1	20	200	200	10	7%-35.7	545.7mg
Starch Glutamate	NSG1	50%	1	20	200	200	10	3%-15.3	525.3mg
	NSG2	50%	1	20	200	200	10	5%-25.5	535.5mg
	NSG3	50%	1	20	200	200	10	7%-35.7	545.7mg
Starch Glutamate+ Crosscarmallose sodium	NSC1	50%	1	20	200	200	10	3%-15.3	525.3mg
	NSC2	50%	1	20	200	200	10	5%-25.5	535.5mg
	NSC3	50%	1	20	200	200	10	7%-35.7	545.7mg

% Cd= drug Concentration in non-volatile solvent, Lf=liquid load factor,
R= Excipient ratio, W= weight of non-volatile solvent, Q= Carrier, q= coating material.

2.2.6. Liquisolid Compact Evaluation:

2.2.6.1. Tablets' physical parameters:

In triplicate, tablets were tested for weight variation, uniformity of tablet thickness and diameter, friability, and hardness[26; 27].

2.2.6.2. Drug content:

The uniformity of drug content was determined as per IP 1996. The tablets were weighed and powered, and 100mg of drug powder was weighed and transferred to a 100ml volumetric flask containing 60ml of ethanol (95%). To dissolve the drug, the flask was shaken, and the volume was adjusted with ethanol. By using ethanol, 10mL of this solution was diluted to 100mL, and the absorbance of resulting solution at λmax of 285nm was measured [28].

2.2.6.3. Disintegration test:

In a suitable vessel, preferably a 1000ml beaker, the assembly was submerged in liquid medium (ED-2L, Electrolab, Mumbai). The liquid volume must be such that the wire mesh is at least 25 mm below the liquid's surface and at least 25mm above the bottom of the beaker at its highest point. A thermostatic arrangement was made for heating the liquid and maintaining the temperature at 37±2°C. The assembly was submerged in a beaker containing 900ml of distilled water, and the apparatus was operate for the duration specified. The tablet's disintegration time was also recorded. Finally, the assembly was taken out from the liquid[28].

2.2.6.4. Dissolution studies:

The dissolution test was used to compare carbamazepine release from liquisolid tablets and mazetol, a marketed tablet. The USP Apparatus 2 (Electrolab, TDT-06L) was used in conjunction with 900ml of 1% sodium lauryl sulphate solution (1 % SLS) at 37±0.5°C, and rotated at 75rpm. After the specified time intervals, a one millilitre sample was withdrawn, and the sink condition was maintained. The samples were filtered, diluted appropriately, and spectrophotometrically analysed at 285 nm wavelength[29].

2.2.6.5. IR- spectroscopy (FTIR):

IR spectrum of Carbamazepine, PEG200, NeusilinUS2 and optimized formulations NSC1 were recorded using an ATR Fourier Transform Infrared Spectrophotometer (MIRacle 10)[22].

2.2.6.6. Statistical analysis:

To determine whether there was a notable difference in the time required for 100% release of carbamazepine from different formulations and the marketed tablet, a one-way ANOVA with Turkey's multi comparison test was used.

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3. RESULT AND DISCUSSION:

3.1. FTIR of carbamazepine

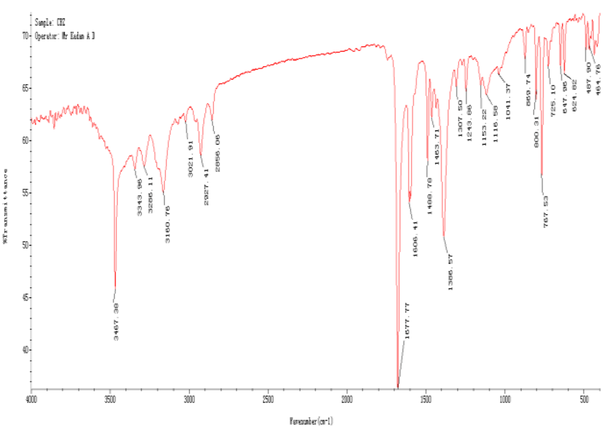

Fig No.1 FTIR Spectrum of Carbamazepine

The FTIR spectra of CBZ showed a characteristic peak at 3467.38 cm^{-1} (-NH_2 vibration), 1677.77cm^{-1} (-C=O vibration), 1606.41cm^{-1} (-C=C vibration).

3.2. Characterization of novel superdisintegrant:

3.2.1. FTIR:

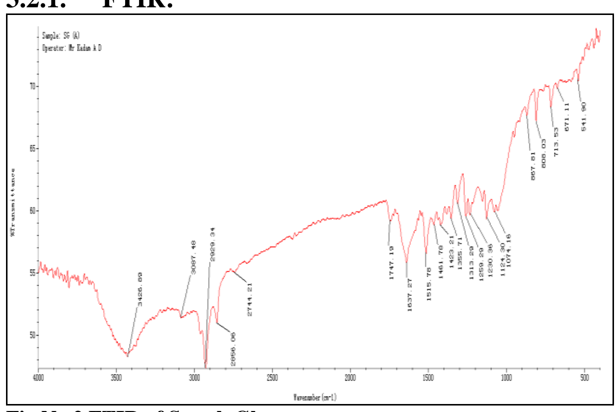

Fig.No.2 FTIR of Starch Glutamate

The FTIR spectrum of starch glutamate revealed a distinct peak at 1637.27cm^{-1} (-R-COO-R′ vibration).

3.3. Solubility studies:

Solubility of carbamazepine in propylene glycol, PEG 200, PEG 400, glycerine and Tween 20 is given in table no.2. Carbamazepine was most soluble in PEG 200 (107.94 mg/ml) and least soluble in Tween 20 (6.84 mg/ml). This is due to the dispersion of a larger fraction of drug in PEG 200, which helps to enhance drug dissolution.

Table No.2 Solubility Data of Carbamazepine

Nonvolatile Solvent	Solubility (mg/ml)
Propylene Glycol	45.10±0.16
PEG 400	68.12±0.29
PEG 200	107.94±0.62
Tween 20	6.84±0.11

3.4. Determination of (ϕ) value:

Relation between angle of slide of carrier and coating material in PEG 200 and corresponding ϕ values is depicted in Fig no.3.

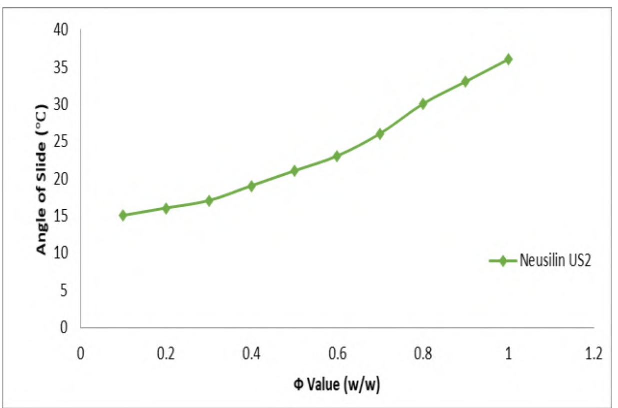

Fig No.3 Liquid Retentions Potential (Φ) of Carrier and Coating Material

3.5. Liquisolid compact evaluation:

3.5.1. Physical parameters of liquisolid compact:

All the physical parameters of liquisolid compact are shown in table no 3. Liquisolid compact containing Neusilin as carrier and coating component showed good compatability, due to its high specific surface area and porosity. Thickness of liquisolid compacts were ranged from 5.33±0.02 to 5.4±0.06557mm and diameter of all the liquisolid compacts was to be in the range of 10±0 to 10.03±0.05744mm as indicated in table no 3. Thickness and diameter of tablet measured by using Vernier caliper.

Tablet hardness test were measured using Monsanto Hardness tester Hardness of tablets was found to be in the range of 3.133±0.05774kg to 3.177±0.04933kg as shown in table no 4.

Due to identical compression force, uniform hardness was achieved.

Tablets were prepared using direct compression method. Because the material was free flowing, uniform weight tablets were obtained as a result of uniform die fill. Tablets were obtained in the 10% acceptable weight variation range as specified by Pharmacopeia. The results are summarizes in table no 3.

Friability of liquisolid compact found to be 0.5129±0.0090 to 0.6003±0.01682% indicated in table no 3. As stated by USP if conventional compressed tablets that loss less than 0.5% to 1% of their weight is generally regarded as acceptable.

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Table No. 3 Physical Parameters of Carbamazepine Liquisolid Compact

Formulation Code	Thickness (mm)	Diameter (mm)	Hardness (kg)	Weight Variation (mg)	Friability %
NCP1	5.4±0.06557	10.03±0.05744	3.17±0.0435	524.9±0.5967	0.5335±0.0205
NCP2	5.35±0.03512	10.03±0.05744	3.133±0.05774	533.8±0.4404	0.5129±0.0090
NCP3	5.4±0.01	10.03±0.05744	3.143±0.0589	544.1±0.4318	0.5458±0.0194
NCS1	5.37±0.03	10±0	3.17±0.0435	574.8±0.4894	0.5591±0.02171
NCS2	5.393±0.02082	10.03±0.05744	3.133±0.05774	587.9±0.3426	0.5716±0.0177
NCS3	5.34±0.02646	10±0	3.177±0.04933	600.5±0.4894	0.5514±0.01322
NSG1	5.383±0.01528	10±0	3.173±0.04619	523.9±0.4286	0.5877±0.02307
NSG2	5.383±0.02517	10±0	3.177±0.04933	534.2±0.3712	0.6003±0.01682
NSG3	5.33±0.02	10.03±0.057444	3.173±0.04619	543.2±0.3401	0.5919±0.02657
NSC1	5.427±0.07024	10.03±0.05744	3.2±0.1	577.4±0.9787	0.5312±0.01649
NSC2	5.367±0.04041	10.03±0.05744	3.177±0.06807	587±0.4648	0.5244±0.0203
NSC3	5.37±0.0435	10.03±0.05744	3.15±0.05196	600.6±0.4686	0.553±0.02453

All values are expressed as mean ± SD (n=3).

3.6. Disintegration time:

Neusilin-crosspovidone batches were failed to disintegrate. Batch NSC1 shows fast disintegration i.e. 42.5±0.5774sec. Starch Glutamate batches shows fast disintegration as compare to crosspovidone batches. Disintegration time of liquisolid compact tablets is given in table no 5 and complies as per IP specifications for all formulated batches except formulations containing Neusilin-Neusilin crosspovidone and these batches were failed to disintegrate. Crosspovidone fails to disintegrate Neusilin-Neusilin compact, for disintegration of this compact addition of 10% fujicalin necessary but it shows more disintegration time. Novel superdisintegrant starch glutamate successfully disintegrate Neusilin-Neusilin compact. Hence Liquisolid compact of Neusilin-Neusilin-starch glutamate-coroscarmellose sodium exhibited fast disintegration.

3.7. Drug content:

The requirement for a steady dose of drug between individual tablets is an essential quality attribute for all pharmaceutical formulations. Uniform drug content was observed for all the formulations given in table no 4. Which is as per the IP specification.

Table No 4. Evaluation of Carbamazepine Liquisolid Formulations

Formulation code	Disintegration Time* (sec.)	% Drug Content
NCP1	No Disintegration	80.81±0.4494
NCP2	No Disintegration	79.87±0.2728
NCP3	No Disintegration	81.3±0.7435
NCS1	91.2±0.05774	94.23±0.4494
NCS2	85.3±0.05774	94.46±0.7784
NCS3	85.2±0.05774	95.11±0.6438
NSG1	71.4±0.05774	95.35±1.61
NSG2	74.4±0.05774	95.35±0.7435
NSG3	78.3±0.1528	96.8±1.189
NSC1	42.5±0.5774	98.8±1.189
NSC2	63.7±0.1	97.04±0.3717
NSC3	60.7±1.155	97.23±0.4494

All data is presented as mean ± SD (n=3).

3.8. Dissolution studies:

The results of in vitro percentage amount of drugs are released at varied intervals of time which is plotted against time to obtain the release profiles and are given in fig no 4.

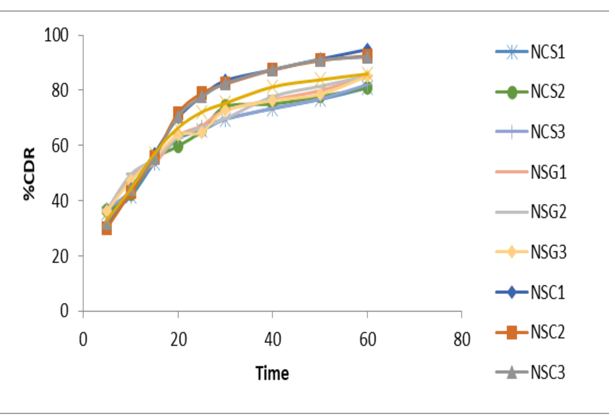

Fig No 4. (A) Dissolution profiles of all batches of liquisolid compact and marketed formulation

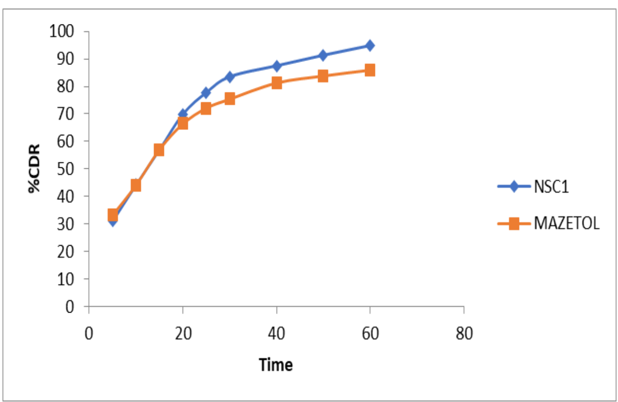

Fig No 4. (B) Dissolution profiles of optimized batches of liquisolid compact and marketed Formulation

Neusilin-crosspovidone showed drug release 80.78±0.5225 to 82.27±1.7121% at the end of 60 minutes. Neusilin-Starch Glutamate, Croscarmellose sodium batch showed drug release 92.27±0.460 to 94.81±0.201% at the end of 60 minutes (shown in table no. 5). Formulations prepared with a novel superdisintegrant demonstrated higher drug release than crosspovidone batches and marketed tablets. As the concentration of crosspovidone increases drug release also increases. Crosspovidone disintegrant failed to

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disintegrate liquisolid compact, but a novel superdisintegrant did, and that batch had an 85% drug release rate. Starch Glutamate showed greater drug release than the marketed formulation. At the end of 60 minutes, the marketed formulation had a drug release rate of 86±0.190%. In Neusilin-Starch glutamate, Croscarmellose sodium batches NSC1 batch shows marked increase drug release than other two NSC2, NSC3 batches.

The fact that the novel superdisintegrant and the drug are already in PEG 200 while being carried by the powder particles may account for the increased dissolve rates of liquisolid compacts when compared to marketed tablet. As a result of the quick disintegration and increased wettability and surface availability to the dissolution liquid, its release is expedited. One of the hypothesised methods for explaining the increased dissolving rate from liquisolid compacts is the compacts' wettability by the dissolution media. PEG reduces the interfacial tension between the dissolution media and the tablet surface, allowing drug particles to wet more easily.

Table No 5. Percentage Amount of Drug Release of Liquisolid Compact

Time	%CDR									
(Min.)	NCS1	NCS2	NCS3	NSG1	NSG2	NSG3	NSC1	NSC2	NSC3	Mazetol
5	35.42± 0.4996	36.58± 0.999	37.1± 0.556	35.54± 0.999	36.63± 0.0999	36.17± 0.995	31.15± 0.915	30.06± 0.993	31.85± 0.1731	33.17± 1.130
10	41.44± 1.044	42.32± 0.875	43.66± 1.117	48.93± 1.964	49.51± 0.9591	47.25± 0.996	44.26± 0.807	42.92± 0.870	44.38± 0.9216	44.16± 0.345
15	53.44± 1.096	54.9± 0.979	54.85± 0.891	53.49± 1.088	55.11± 1.271	55.56± 0.991	57.07± 0.466	55.85± 1.723	55.63± 1.947	57.08± 0.523
20	62.39± 0.096	59.87± 0.8834	63.34± 0.2567	63.42± 1.362	62.97± 0.997	63.88± 0.697	69.96± 0.236	71.85± 0.6351	70.71± 2.058	66.45± 0.156
25	65.33± 1.467	65.34± 0.4066	66.81± 0.2581	67.17± 0.8298	66.43± 0.956	64.87± 0.984	77.79± 0.343	79.05± 0.113	77.67± 1.714	72.13± 0.347
30	69.38± 0.2671	74.23± 0.428	69.83± 0.177	72.78± 0.3209	69.74±± 0.956	72.72± 0.986	83.52± 0.550	82.42± 0.965	82.6± 1.144	75.41± 0.583
40	73.28± 0.1788	75.21± 0.2835	73.73± 0.33646	76.53± 0.9379	77.67± 0.247	76.41± 0.219	87.5± 0.454	87.55± 0.368	87.67± 0.5245	81.3± 0.762
50	76.56± 0.09445	77.93± 0.9728	77.07± 0.2701	79.83± 0.4464	81.44± 0.134	78.72± 0.264	91.32± 0.527	90.96± 0.5372	91.14± 1.364	83.87± 0.206
60	80.78± 0.5225	81.12± 0.6438	82.27± 1.7121	85.04± 0.8721	85.08± 0.974	85.32± 0.969	94.81± 0.201	92.67± 0.1041	92.27± 0.460	86± 0.190

All values expressed as mean ± SD (n=3).

3.9. IR- spectroscopy:

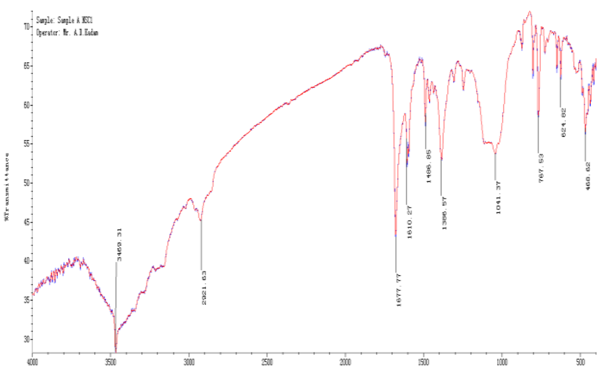

Fig No.5 FTIR Spectrum of Optimized Formulation

The continuous several range of CBZ 3470- 1600cm^{-1} and 1619.29cm^{-1} represent the ammonia and ester group respectively present in the batch NSC1.

However, comparison of the spectra demonstrated no new characteristic peaks in the liquisolid compact formulation which indicated no physical or chemical interaction between CBZ and Starch Glutamate are given in Fig No. 5.

3.10. Statistical analysis:

There was no significant difference ($P < 0.05$) between the release profiles of the marketed tablet and liquisolid compacts, according to the results of a one-way ANOVA with Turkey's multi comparison test.

4. CONCLUSION:

The results indicated that, liquisolid compacts of CBZ can be prepared using a novel superdisintegrant such as Starch Glutamate. To ensure the safety of newly developed superdisintegrants, the base of synthesis was an endogen amino acid (Glutamic acid), and starch was successfully derivatized with Glutamic acid. The comparison of Starch Glutamate, Croscarmellose Sodium, and Crosspovidone-made tablets revealed that starch Glutamate and Croscarmellose Sodium-made tablets had better disintegration and dissolution behaviours than the others. It was necessary to pick superdisintegrants to maximise drug dissolving in a time when formulators were confronted with a growing number of poorly soluble drugs. The effects of Starch Glutamate, Croscarmellose Sodium, and Crosspovidone on the dissolution rates of poorly soluble drugs were investigated, and it was discovered that Starch Glutamate, Croscarmellose Sodium has the fastest

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dissolving rate. The drug release of a Liquisolid compact formulation with Starch Glutamate superdisintegrant was higher than that of other disintegrants and marketed tablets. This study came to the conclusion that Starch Glutamate might be used as a new superdisintegrant in the pharmaceutical industries.

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DEGRADATION STUDY OF DIFFERENT BRANDS OF ANTIPYRETIC TABLETS BY UV SPECTROSCOPY

Umesh Rangat*, Anjan Ladage, Bharatee Chaudhari and Vivekkumar Redasani

YSPM'S, Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara.

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***Corresponding Author**
Umesh Rangat
YSPM'S, Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara.

ABSTRACT

In the present project forced degradation study of different brands of Paracetamol Tablets were performed. Forced degradation of drug substance was performed by exposing it to Acid, Base, and UV light. The amount of degradation product can be determined with the help of UV spectrophotometer. Forced degradation of drug substance of Pacimol, Pyrigesic and Calpol were observed. In the result negligible difference was observed on exposure to UV. This method can be used successfully for studying the stress degradation factors. Because this method is less time consuming and simple and cost effective. Three different Brands that is Pacimol, Pyrigesic and Calpol of Paracetamol Tablets were used for the study. It was found that very negligible degradation was occurred.

KEYWORDS: UV spectroscopy, Paracetamol, UV Cabinate, Acid Degradation, Base Degradation.

INTRODUCTION

One of the most common symptoms is pain and this is one of the most frequent reasons why people seek medical care. Therefore, it is not surprising that the analgesics are among the most widely used categories of drug. Hence, for the treatment of inflammation and pain Paracetamol is used. Chemically Paracetamol is 4-hydroxyacetanilide. Paracetamol is a weak peripheral cyclooxygenase inhibitor and from the inhibition of prostanoid synthesis in the central nervous system, analgesic effect of Paracetamol may arise. Antipyretic effect of Paracetamol is reported to inhibit prostaglandin synthesis at the level of the hypothalamus causing alteration in body temperature.[1]

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The Principle of UV-Visible Spectroscopy is based on the absorption of ultraviolet light or visible light by chemical compounds, which results in the production of distinct spectra. Spectroscopy is based on the interaction between light and matter. When the matter absorbs the light, it undergoes excitation and de-excitation, resulting in the production of a spectrum. When matter absorbs ultraviolet radiation, the electrons present in it undergo excitation. This causes them to jump from a ground state (an energy state with a relatively small amount of energy associated with it) to an excited state (an energy state with a relatively large amount of energy associated with it). It is important to note that the difference in the energies of the ground state and the excited state of the electron is always equal to the amount of ultraviolet radiation or visible radiation absorbed by it. In many laboratories, spectrophotometric method was used due to less equipment cost and economical maintenance advantages. By the help of this technique, the UV absorbance spectra are measured at 200–400 nm. In accordance with the International Conference on Harmonization guideline, the force degradation state of active pharmaceutical substance includes acidic, basic and photolytic conditions. We already performed these types of degradation studies which are useful for pharmacy profession. Basic parameters for drug degradation studies are acid/base stress testing, humidity and with temperature, photo degradation. Forced degradation of drug was performed with acidic, basic and Uv light condition. Forced degradation of drug substance in UV light was performed by exposing the drug substance to UV light.[2]

MATERIALS

For the present project work different paracetamol tablets brands were used such as Calpol 500 mg tablets of GlaxoSmithKline Pharmaceutical Limited, Pyrigesic 500 mg tablets of East India Pharmaceutical Works Limited, Pacimol 500 mg tablets of Ipca Laboratories Limited and Febrex Indoco Remedies Limited. 1M NaOH, 1M HCL, reference standard Paracetamol, Pyrex type stirrer, measuring cylinder, pipette, funnel, beaker and volumetric flask, petri dish, cuvettes, butter paper, Whatman filter paper No. 44, spatula, tissue paper were used. Freshly laboratory prepared distilled water was used to wash glasswares. Weighing Balance, Shimadzu UV spectrophotometer.

METHOD

Preparation of 1mole/liter NaOH

In 100 ml volumetric flask, accurately 4 g NaOH was dissolved and to make up the volume up to 100 ml, deionized water was added.[3]

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Preparation of 1 mole/liter HCl

A total of 8.36 ml hydrochloric acid (37% 12 mol/L) was took accurately analytical grade in 100 ml volumetric flask to make up the volume up to 100 ml by adding deionized water. [4]

Preparation of paracetamol stock solution (API)

1. Weigh accurately 100 mg of Paracetamol IP pure powder and add 15 ml of 0.1 N NaOH and dilute up to 100 ml with distilled water (1000 ug /ml).
2. Prepare 6 standard dilutions from above solutions by diluting 0.5 ml, 1 ml, 1.5 ml, 2 ml 2.5 ml, 3 ml to 100 ml with distilled water.
3. Run these 6 standard dilutions of 5, 10, 15, 20, 25 and 30 ug/ml concentration for absorbance against blank. Plot calibration curve between absorbance versus concentration at 257 nm.
4. Find out equation of line i.e Y= mx+c. [5]

Preparation of Tablet (Test) solution

Weigh the 10 tablets of different brand of Paracetamol with the help of clean and dry mortar and pestle Calculate average weight of powder. Take Powder was equal to 20 mg of Paracetamol of each brands. Paracetamol powder which is equal to Calpol (25.44mg), Pacimol (22.68mg), Pyrigesic (22.72) were accurately weighed. In the 100 ml volumetric flask, all of 3 brands powders transferred individually. These powder samples were dissolved and shaked with water and finally make up the volume up to 100 ml respectively for each sample. A total of 20 mg/100 ml concentration solution was preferably obtained. By using spectrophotometer at 257 nm wavelength individually all brands absorbance were determined.[6-10]

Procedure for forced degradation studies

1. For acid

Forced degradation of drug substance in acidic media was performed by taking 5 ml of 20 mg/100 ml of Pyrigesic, Pacimol and Calpol in 3 separated testtubes, then 5 ml of 1 mol/L HCl was added in each test tube. The sample was left for 30 min. Solution was transferred to a separated cuvette after the time period completion and UV absorbance of the solution was measured at the 257 nm wavelength.[6-10]

2. For base

Forced degradation of drug substance in basic media was performed by taking 5 ml of 20 mg/100 ml solution of Pyrigesic, Pacimol and Calpol in 3 separated test tubes, then 5 ml of NaoH was added in each test tube and the sample was left for 30 min, and then UV absorbance of solution was measured at 257 nm wavelength.[6-10]

3. For UV light

Forced degradation of drug substance in UV light was performed by taking the 5 ml of 20 mg/100 ml solution of Pyrigesic, Pacimol and Calpol, then 5 ml of water was added in each test tube and these test tubes were exposed to UV light for 30 min, and then UV absorbance of solution was measured at 257 nm wavelength.[6-10]

RESULT

Preparation of callibration curve

Table 1: Absorbance of standard paracetamol solution.

Sr. no.	Standard Concentration	Absorbance
1	0	0
2	5	0.0600
3	10	0.1204
4	15	0.1779
5	20	0.2105
6	25	0.3155
7	30	0.3685

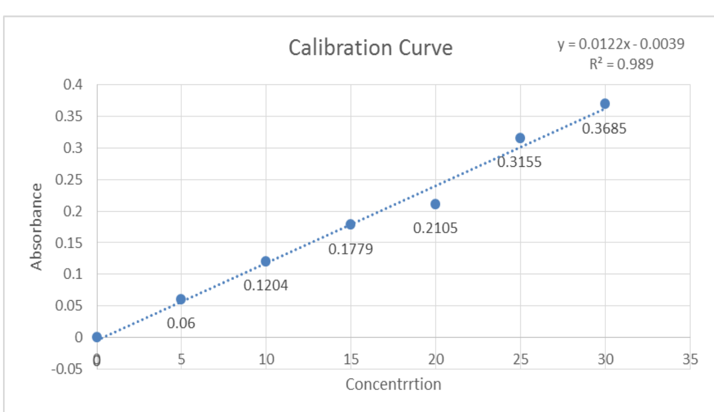

Fig. no. 1: Callibration curve of standard paracetamol solution.

Forced degradation studies

The degradation study was conducted on three brand of Paracetamol which are Pyrigesic 500 mg tablets of East India Pharmaceutical Limited, Pacimol 500 mg tablets of IPCA Laboratories Limited and Calpol 500 mg tablet of Glaxosmithkline Pharmaceutical Limited.

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When Paracetamol brands were treated with the 1 mol/L HCL, it showed small degradation. When Paracetamol brands were treated with the 1 mol/L NaOH drugs, it showed degradation. When exposed to UV light showed more degradation. Table 1 represents the UV absorption of different brands of the Paracetamol before and after exposing to the degradation environment. We concluded according to our results that when the Calpol introduced into acidic medium 1 mol/L HCL, it showed degradation that is (78.11%) Pacimol showed degradation in acidic medium that is (71.71%) Pyrigesic also gave greater results on exposure to acidic medium (70.63%) respectively. Similarly on exposure to 1 mol/L NaOH basic medium, the Calpol showed the (82.91%) degradation whereas Pacimol showed degradation to minor extension that is (74.62%) while Pyrigesic gave moderate results on exposure to basic medium (69.36%) respectively. When Calpol (52.83%), Pacimol (59.51%) and Pyrigesic (61.98%) exposed to UV light for 30 min and evaluated for degradation studies, it also showed minor changes in concentration respectively for degradation studies. Results of degradation studies are given in Tables 2.

Table 2: Absorbance of different brand of paracetamol.

Tablet	Treatment	1	2	3	Average	Percentage
Calpol	Before	2.4436	2.4436	2.4559	2.4477	84.93%
	Acid Treatment	2.2518	2.2924	2.3098	2.2846	78.11%
	Base Treatment	2.3872	2.3872	2.3979	2.3907	82.90%
	UV Treatment	1.7423	1.7423	1.7328	1.549	52.83%
Pacimol	Before	2.2291	2.2441	2.2291	2.2341	77.30%
	Acid Treatment	2.0555	2.0861	2.0911	2.0775	71.71%
	Base Treatment	2.1549	2.1611	2.1611	2.1590	74.62%
	UV Treatment	1.7328	1.7328	1.7423	1.7359	59.51%
Pyrigesic	Before	2.0705	2.0915	2.0915	2.0845	71.96%
	Acid Treatment	2.0362	2.0457	2.0604	2.0474	70.63%
	Base Treatment	2.0087	2.0087	2.0177	20117	69.36%
	UV Treatment	1.8124	1.8013	1.8013	1.8005	61.98%

DISCUSSION

In the present project work all the brands of Paracetamol were exposed to different degradation parameters, there was small degradation in the active ingredient of the brands of Paracetamol. The brands i.e. Calpol, Pacimol and Pyrigesic when they come in contact with different degradation parameters (before, acid, base, and UV) showed degradation of drug substance. According to specification of United State Pharmacopoeia, the content official limit of not less than (98%) and not more than (101%) the labeled amount. We have

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concluded from our studies that Paracetamol less degrades in acidic and basic medium as compared to UV light treatment.

CONCLUSION

In the present project degradation study was performed as per ICH guideline. Paracetamol tablets were expose at different condition like Acid, Base and UV light and it was found that some amount of degradation was occurred at different conditions. In Calpol tablet degradation study it showed degradation in UV treatment more prominent. Similarly Pacimol and Pyrigesic also showed more degradation in UV treatment as compared to Acid and Base treatment.

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CLEANING VALIDATION OF TABLET COMPRESSION MACHINE BY SWAB SAMPLING

Poonam D. Khalate*, Pratiksha S. Londhe, Bharatee P. Chaudhari, Vivekkumar K. Redasani

YSPM'S, Yashoda Technical Campus, Wadhe, Satara.

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***Corresponding Author**
Poonam D. Khalate
YSPM'S, Yashoda Technical Campus, Wadhe, Satara.

ABSTRACT

Validation of cleaning technique using swab sampling and application in determining residual L-ascorbic acid in production area equipment, as well as confirmation of cleaning procedure efficiency. After producing L-ascorbic acid 10 mg chewable tablets, the swab sampling and UV method for residual determination of in L-ascorbic acid swab samples from equipment surfaces were established and verified. For L-Ascorbic Acid in tablet form, a unique, safe, and sensitive method of spectrophotometric measurement in the UV region has been devised. Water as diluents were used to develop and validate the technique for L- Ascorbic Acid.

KEYWORDS: Cleaning Validation, Linearity, Swab, Absorbance, UV Spectroscopy, L-Ascorbic Acid.

INTRODUCTION

Validation is documented evidence that a planned procedure will function consistently according to the previously stated results. Process validation is defined by the quality systems regulation as establishing, by objective evidence, that a process consistently generates a result or product that meets its pre-determined specifications. A quality system's purpose is to generate products that are suitable for their intended usage on a consistent basis.

Cleaning validation is proof that an approved cleaning operation will result in equipment that is suitable for the processing of pharmaceutical products. Cleaning pharmaceutical equipment is becoming increasingly important in terms of regulatory compliance. To evaluate a cleaning procedure, a sample of the product contact surfaces of the equipment must be

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taken to determine the level of residuals present. Swab sampling, rinse sampling, coupon sampling, placebo sampling, solvent sampling, and product sampling are the six sampling procedures. It's critical to use the right procedure for detecting residue in the cleaning sample. The test method used to validate the cleaning procedure should be able to precisely quantify the concentration of any substances of interest that may be present in the sample. UV spectroscopy, HPLC, GC, HPTLC, atomic absorption spectroscopy, fluorimetry, and simple photometry are some of the specialised analytical procedures utilised for cleaning validation. Visual examination, gravimetric analysis, pH, conductivity, microscopy, titration, and the total organic carbon method are examples of non-specific analytical procedures often used for cleaning verification. The accuracy, precision, linearity, specificity, range, and LOQ/LOD would all be included in the method validation SOP. The scientific basis for cleaning validation is usually mentioned in the protocol's limit section. The scientific rationale for the real restriction should be logical, thorough, and simple to comprehend.

Fibrous material is used in the swab sampling method to swab a surface in order to collect samples. Direct surface sampling method is another name for the sample technique. Textiles that are woven or nonwoven and have a plastic handle make up the fibre component of swabs. This approach involves pre-wetting a swab (i.e., a fibrous substance) with a suitable solvent before sampling a residue that is soluble in that solvent. Prior to sampling, the edges of the swab head are typically squeezed against the walls of the vial or test tube to eliminate excess solvent. This is important because too much solvent may leave interesting extractable materials on the surface, acting as a source of residues and producing inconsistent findings. A suitable extraction solvent is used to extract the analyte that needs to be analysed and measured from the swab head after sampling has been completed across a predetermined area. The extracted solvent may be the same as or different from the solvent used to moisten the cotton swab.

Swab sample recovery should be regarded as a serious concern since studies on swab recovery show that if a residue is present on an equipment surface, it may be accurately assessed and quantified by using analytical methods and sampling techniques. Developing a consistent level of recovery from the equipment surface is the goal of the scientific approach known as a recovery study. It should be demonstrated that recovery is feasible from all product contact materials sampled in the apparatus using all the sampling techniques. The size, shape, and characteristics of the swab head, as well as the characteristics of the swab

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handle (such as flexibility and length), all affect the recovery of residues from surfaces. Sampling recovery studies are lab experiments using coupons of equipment made of various construction materials (such as stainless steel, glass, PTFE, and EPDM) that have been sampled and have been contaminated with residues to be analysed. The normal range of acceptable variation for recovery outcomes at a single spiking level is between 15% and 30% RSD. One would prefer to recover 100% of the challenge, however depending on the sample conditions and the residue, recoveries may only be restricted to 75% to 80%.[1,2]

L- Ascorbic Acid

Molecular formula	-	$C_6H_8O_6$
Molecular Weight	-	176.12
IUPAC Name	-	(5R)-[(1S)-1,2-Dihydroxyethyl]-3,4-dihydroxyfuran-2(5H)-one
Appearance	-	White or light-yellow crystalline powder
Melting Point	-	190^0 C
Boiling Point	-	553^0 C
Soluble in	-	Water, Glycerol, Ethanol, Polypropylene glycol

Vitamin C is another name for it. L-ascorbic acid is a water-soluble vitamin that can be found in citrus and other fruits and vegetables, as well as being purchased as a dietary supplement. Scurvy is a disease that can be prevented and treated with this supplement. L-ascorbic acid is a vitamin that helps with tissue healing, collagen creation, and the enzymatic manufacture of some neurotransmitters.

Ascorbic acid is a water-soluble vitamin that is found in nature (Vitamin C). Ascorbic acid is a powerful reducing and antioxidant that aids in the battle against bacterial infections, detoxification, and collagen production in fibrous tissue, teeth, bones, connective tissue, skin, and capillaries. Vitamin C is found in citrus fruits and vegetables and cannot be synthesised or stored by humans, thus it must be taken through the diet.

L-ascorbic acid is a crystalline powder that is white to pale yellow in colour and has a nice sharp acidic taste. It is almost odourless.[3,4]

❖ MATERIALS AND METHODS

L- Ascorbic acid (Reference std.) by S. D. Lab, Distilled water by Distillation Unit of Equitron, Texwipe swab sticks by Somya Digital Technologies New Delhi, Tablet Compression Machine supplied by Nikhil's Scientifics, UV Spectrophotometer (Systronics).[1,4]

❖ Selection of Analytical Performance Characteristics

The analytical performance criteria listed below were chosen for use in analytical technique validation for cleaning swab samples: Maximum detection, Blank swab interference analysis, Linearity, and Range, Precision, and LOD and LOQ, as well as drug recovery from spiked SS plates (accuracy).[4,5]

❖ ANALYTICAL METHOD VALIDATION

1. Detection of λ Maxima for L- Ascorbic acid

Weighed accurately 100mg of working standard and transferred into 100 ml volumetric flask and made up the volume with water to 100 ml. 1 ml of above solution was taken in another 100 ml volumetric flask and made up the volume to 100 ml with water (10ppm).

2. Blank Swab Interference Analysis

Blank swab solution

6 Texwipe swabs were placed in various stopper test tubes with 100 ml water. After 2 minutes of sonication, the swab was withdrawn from the sample solution. A homogeneous solution was prepared. By scanning a 10 ppm standard solution, the absorbance of the standard solution, blank solution, and blank swab solution was determined.

The purpose of this investigation was to see if the swab and cleaning agent interfered with the absorbance of L- Ascorbic acid at the maxima.[1,4]

3. Linearity

Prepared the test solution using L- Ascorbic acid working standard at concentration level 2, 4, 6, 8 and 10 ppm. Measured the absorbance of all solutions at determined maximum

wavelength in duplicate. Plot the graph between absorbance (y-ordinate) and concentration (x-abscissa) and determined the regression and correlation coefficient (r^2).[1,5,6]

4. Precision

Repeatability of Measure of Absorbance

To check repeatability, measurement of absorbance 10 ppm solution of drug was measured 6 times at λ max and % RSD was calculated.[4,5]

- **Reproducibility of Measurement of Absorbance**

The absorbance of different concentrations was measured in three replicates to ensure repeatability.

- **Intraday analysis**

Intraday analysis was determined by analyzing the 5 drug concentration in triplicate at same day and % RSD was calculated. In this study 2,4,6,8 and10 ppm solution was prepared in triplicate form and the absorbance was determined at λ max of drug.[1,5,6]

❖ **Interday Analysis**

Inter day precision is determined by analyzing drug daily for three days.[7,8]

5. Recovery of drug from Spiked SS Plates

Determined the recovery of the method by applying the method to SS plates to which known amount of analyte (L- Ascorbic acid) has been added.

Spiking with solution of 50, 100 and 200 ppm

Spiked uniformly three 5 X 5 cm^2 three separate SS plate (316 SS grade) with 400 µl solution of 50, 100 and 200 ppm standard solutions respectively with the help of a micro pipette and allowed the surfaces to dry. This procedure was performed in triplicate.

Test solutions

Took out the swabs from stoppered test tubes and squeezed the excess swabbing solvent by pressing it with the wall of the test tube. Swabbed the dried spiked plate as per procedure of sampling of swab on equipment (vertically and horizontally). Using single swab from one spiked location (plate). Placed this spiked swab in stopper test tube containing 10 ml of water and sonicated the test tube for about 10 min. to extract the drug in solution. Swabbed at 9 plates (3 concentrations in triplicate). Measured the absorbance of each of the swab test

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solutions at determined λmax. Compared all these swabbed solutions of 50, 100 and 200 ppm with standard solution of 2, 5, 10 ppm and % recovery should not be less than 80 %. A recovery of >80% is considered good, >50% reasonable and, <50% questionable. The amount of drug found in test solution was calculated by determining concentration of drug in test solution.[8,9]

6. Limit of Detection

Preparation of standard solution (10 ppm)

Took 100mg of drug (working standard) to 100 ml volumetric flask and made up to 100 ml with water. 1 ml of this solution was taken in 100 ml volumetric flask and made up 100 ml with water. From stock solution prepared 100 ml each of 0.1, 0.2, 0.3, 0.4, 0.5 ppm by suitable dilutions.[10]

Using the following formula, calculate the LOD using the absorbance of all of the above concentrations:

$$LOD = 3.3\ \sigma / S$$

Where,

σ- Standard deviation of response

S- Slope of calibration curve

7. Limit of Quantitation

Determined the concentration at which the analytical method can quantify the analyte using the absorbance obtained from the Limit of Detection. The following formula can be used to determine LOQ.[10,11]

$$LOD = 10\sigma / S$$

Where,

σ- Standard deviation of response

S- Slope of calibration curve

➢ TABLET COMPRESSION MACHINE

❖ Selection of swab sampling points of equipments

Following are the swab sampling point for the cleaning validation

A. Granulation Area: Vibro Sifter, Multimill, Rapid Mixer Granulator, Fluidised Bed Drier, Bin Blender, IPC, Tipper

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B. Compression Area: Compression Machine, Deduster (LHS), Metal detector (LHS), Deduster (RHS), Metal detector (RHS)

C. Inpection Area: Tablet capsule sorter,

D. Coating Area: Auto coater, Roll Compactor

F. Packaging Area: Blister Packing Machine[1,4,12]

❖ Procedure of swab sampling from the equipment

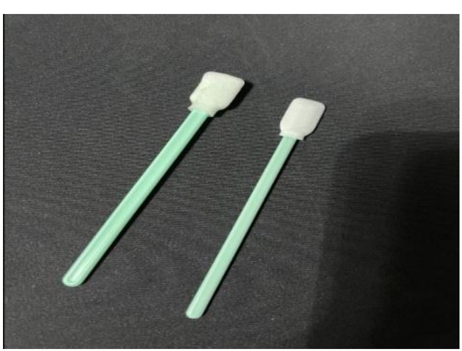
Swab samples were collected from the different locations of the equipments

• Swab samples was done in the following manner Sampling area = 5 X 5 cm = 25 cm2.

5×5 cm^2 Sterile Plastic Swabbing Template

❖ Sampling patterns

As illustrated in the diagram, wiped the defined area in both directions. Applied only one time. The surface was not rubbed in to and forward movement. Swabbed the specified area and stored in a test tube containing 10 ml of water then stoppered the test tube. This sample was then analyzed by the UV spectrometer.[4,13]



Swab Sticks

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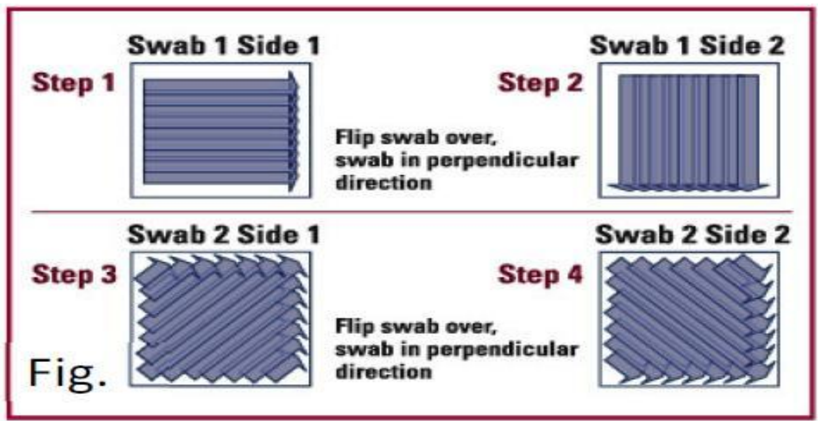

Sampling Pattern

- **Procedure for Analysis of the Samples**

Preparation of standard solution (10 ppm)

About 100mg of L- ascorbic acid was taken in 100 ml volumetric flask. Then made up with water and placed in sonicator to prepare uniform solution. Took 10 ml of this solution in 100 ml volumetric flask and made up with water. Took the absorbance in UV spectrophotometer. Preparation of sample solution swab is placed in a test tube filled with 10 ml of water and sonicated for 10 min. Took the absorbance of solution in UV spectrophotometer. The amount of drug present in each swab was calculated.[14]

- **Optimization and validation of cleaning procedure for L- ascorbic acid tablets on compression machine**

Cleaning validation was performed to demonstrate that the cleaning technique was effective for residues according to the predetermined L-ascorbic acid acceptable limit in the equipment train.[6,9,15]

- **Sampling procedure**

After cleaning, a swab sampling process was utilised to determine the amount of drug residue left in the equipment. Sterile cotton swabs with a polypropylene swab stick were utilised in an HDPF container for this procedure. Swabs were immersed in distilled water and saturated for 15 minutes before being used to collect samples. A 5 cm × 5 cm swab area was chosen for each sampling location and swabbed according to the swabbing pattern. The pattern of swabbing and pressure applied was such that it collected the maximum residue present in the selected area. All this operation was done with care and wearing powder free sterile gloves in hands. The swab wiped from selected area was placed in the sterile HDPF containing 10 ml

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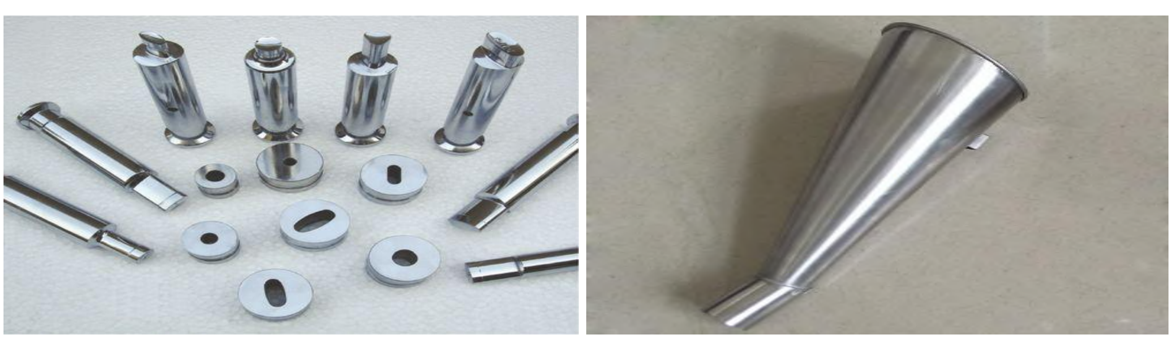
of distilled water and capped securely. Each tube was sonicated for 5 min; the extract was collected and analyzed.[4,11,16]

❖ Sampling locations

It is important to include swab samples from the equipment's most difficult to clean and worst-case locations, but the sampling locations were chosen to be representative of all sections of the equipment, including easy-to-clean surfaces.

The given piece of equipment had a 99 percent surface that was easily accessible, cleanable, and eye catching. Only 1% or less of the equipment surface was difficult to clean, hence it was presumed that the equipment was as filthy as the hard-to-clean surface samples.[17]

Tablet Compression Machine



Dies and Punches **Feeding hopper**

Outlet Hopper **Tablet Scraper**

❖ **Cleaning procedure**

After compressing the L-ascorbic acid 10 mg tablets, the tablet machine was cleaned according to industry standard procedures for cleaning pharmaceutical equipment. Cotton swabs were used to collect the samples, which were wiped over a 5 cm5 cm region of predetermined sampling locations. The samples were evaluated and compared to the set of acceptance criteria.

Following steps were followed in the cleaning procedure of the tablet machine after compression of the L- Ascorbic Acid 10 mg tablet.

- The machine's main power supply was turned off.
- The pressure that was exerted to the machine's roller during compression was released.
- Powders, containers, and tablets from previous batches were removed from the producing area.
- A dry towel was used to remove the loosely stuck powder from the machine.
- Compression machine was dissembled as follows:
- Unscrew the top screws to remove the hopper from the spindle.
- The door of machine were opened.
- By unscrewing the upper screws, the feed frame was removed from the die table.
- The tablet discharge chute was removed from the machine after the screws were loosened.
- Washing was performed on all of the above-disassembled pieces.
- The machine's bottom side cover was removed.
- The higher punches were removed by removing the upper punch guide and using the flying wheel to rotate the turret.
- Using an allen key, the turret's die locking screw was released, and dies were removed by pressing through the bottom guide hole with the use of a die driving road.

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- Cleaning of die and punches
- With the help of the dry towel, the adhering powder was removed from the punches.
- Clean wet towels dipped in purified water were used to clean punch bowls.
- Wet cloths dipped in purified water were inserted into the hole to clean the dies. A moist cloth dipped in purified water was used to wipe the outside of the dies.
- After a visual check, cleaned punches and dies were stored in the proper cabinet.
- A clean, wet towel dipped in purified water was used to clean the die lock.
- After cleaning, the die lock was let to air dry.
- Cleaning of machine
- A wet cloth dipped in purified water was used to clean the machine's top base, upper roller, upper cam track, and bottom track, followed by a dry cloth clean.
- The vacuum cleaner was used to remove all adherent material from the turret, which was then wiped clean with a clean cloth soaked in water before being cleaned with a clean cloth soaked in IPA.
- A dry clean towel was used to clean the upper punch shank, lower punch shank, and die pocket.
- A clean dry cloth was used to wipe the machine's non-contact parts.
- Doors were cleaned with a purified water-soaked cloth followed by a dry clean cloth.
- Washing
- To remove adherent materials on SS surfaces, the hopper, feed frame, liner removal plate, and discharge chute were flushed with purified water for at least 4 minutes.
- Final rinse and drying
- Finally, the feed frames, hopper from the inside, liner, and discharge chute were rinsed with purified water for no more than 4 minutes. The excess water was wiped away with a dry, clean cloth.
- Items were carried to the machine area once they had been cleaned.
- The machine was marked as "ready to use."[1,5,7,18]

RESULT AND DISCUSSION

1. Detection of λmax of L- Ascorbic acid

The 10 ppm solution was scanned and λmax was found to be 295.4 nm.

2. Blank swab interference

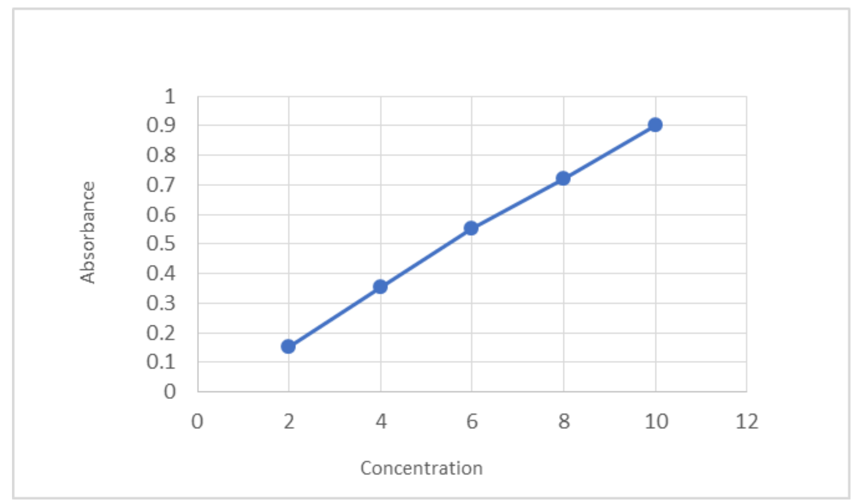
This study was performed to check the interference on the absorbance of L- Ascorbic acid at the maxima due to swab and cleaning agent.

Solutions	Standard solution	Swab Solution 1	Swab Solution 2	Swab Solution 3	Swab Solution 4	Swab Solution 5	Swab Solution 6
Absorbance at 295.4 nm	0.8996	0.0008	0.0012	0.0005	0.0011	0.0010	0.0017

3. Linearity

Linearity of the analytical method was its ability to elict test result that are directly proportional to concentration of the drug substance taken for test, within a given range of 2-10 ppm.

Conc. (ppm)	2	4	6	8	10	Correlation coefficient	Intercept	Slope
Absorbance	0.1522	0.3542	0.5526	0.7158	0.8999	0.9992	-0.0221	0.0928



4. Precision

Precision is the measure of either degree of reproducibility or repeatability of analytical method. It is expressed as standard deviation or coefficient of variance.

Sr. No	1	2	3	4	5	6	Mean+ Std. Deviation	% RSD
Absorbance	0.8952	0.8999	0.9002	0.8970	0.8986	0.8996	0.8988+ 0.8993	100.05

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Reproducibility of absorbance

- **Intraday analysis**

Intraday analysis was determine by analyzing drug as per procedure for three times in the same day.

Sr. No	Conc. (ppm)	Absorbance			Mean	Standard Deviation	% RSD
1	2	0.1519	0.1523	0.1522	0.1521	0.00020	0.1368
2	4	0.3541	0.3536	0.3545	0.3540	0.00045	0.1273
3	6	0.5529	0.5531	0.5529	0.5529	0.00011	0.0208
4	8	0.7162	0.7158	0.7165	0.7161	0.00035	0.0490
5	10	0.8991	0.8999	0.9001	0.8997	0.00052	0.0588

- **Interday analysis**

Interday precision was determined by analyzing drug as per procedure daily for three days. Reproducibility was evaluated by coefficient of variation.

Sr. No	Conc.(ppm)	Absorbance			Mean	Standard Deviation	% RSD
		Day 1	Day 2	Day 3			
1.	2	0.1569	0.1601	0.1612	0.1593	0.0022	1.4014
2.	4	0.3521	0.3451	0.3496	0.3489	0.0035	1.0166
3.	6	0.5495	0.5461	0.5529	0.5494	0.0034	0.6187
4.	8	0.7222	0.7124	0.7162	0.7169	0.0049	0.6891
5.	10	0.8999	0.9011	0.9021	0.9010	0.0011	0.1222

5. Recovery of drug from Spiked SS Plates

Recovery study was performed by spiking the different concentration solution on SS plate and then swabbed by swab sticks. Then analysis in UV and compared the absorbance of test solution with standard solution results.

Conc.	Abs. 1	Abs. 2	Abs. 3	Mean	Std. abs	Conc.(ppm)	% Recovery
50	0.1502	0.1255	0.1519	0.1425	0.1522	18.5	92.5
100	0.4132	0.4267	0.4293	0.4230	0.4409	35.9	89.7
200	0.8581	0.8473	0.8575	0.8543	0.8999	71.3	88.7

6. Limit of Detection

It was calculated based on standard deviation of response and slope of calibration curve

Conc. (ppm)	Absorbance		Mean absorbance
	Absorbance 1	Absorbance 2	
0.1	0.0108	0.0109	0.0108
0.2	0.0221	0.0219	0.0220
0.3	0.0301	0.0305	0.0303
0.4	0.0472	0.0469	0.0470
0.5	0.0525	0.0524	0.0524

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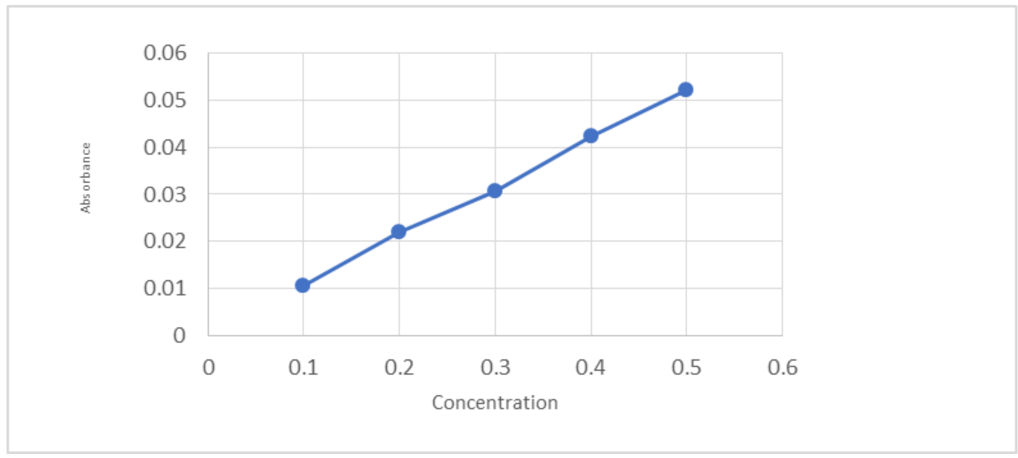

Took the absorbance of all the above concentration and calculated the LOD by using following formula:

$$LOD = 3.3\ \sigma / S$$

Where,

σ- Standard deviation of response

S- Slope of calibration curve

LOD = 3.3× 0.0172 / 0.1082

= 0.0570 / 0.1082

= 0.5143 µg/ml

7. Limit of Quantitation

LOD = 10× 0.0172 /0.1082

= 0.1727 / 0.1082

= 1.5957 µg/ml

➢ **Tablet Compression Machine**

L- Ascorbic acid tablets 10 mg were manufactured in order to test the cleaning procedure on the tablet machine. The purpose of this validation was to demonstrate the cleaning procedure's effectiveness and uniformity. Validation was also carried out in order to meet the development method's regulatory requirements. After production, tablet compression machine was subjected to above cleaning procedure. The results of the visual and chemical inspections were documented.[4,8,18]

Sr. No	Sampling Point	Absorbance	Concentration
1.	Turret	0.0109	0.35
2.	feeding Hopper	0.0016	0.24
3.	Outlet Hopper	0.0080	0.25
4.	Dies	0.0054	0.29
5.	Punches	0.0102	0.34

CONCLUSION

Cleaning procedure was optimized for determination of drug residues on different parts of tablet machine in different steps of cleaning. On the tablet compression machine, cleaning validation was performed. L- Ascorbic acid tablets were formulated for cleaning validation study. The drug's chemical residue was detected within the predetermined acceptance parameters at various areas of the equipment surfaces.

To show cleaning validation, swab sampling and the UV method were devised and validated for quantifying L- Ascorbic acid residues on stainless steel surfaces of plant equipment following manufacturing of L- Ascorbic acid 10 mg chewable tablets. Selective, accurate, precise, and linear methods with the suitable swab wipe approach were discovered.

The swab sampling and UV approach can be successfully applied in cleaning validation for quantitative measurement of L- Ascorbic acid residues following the manufacture of L-Ascorbic acid chewable tablets in different pharmaceutical quality control laboratories.[18,19]

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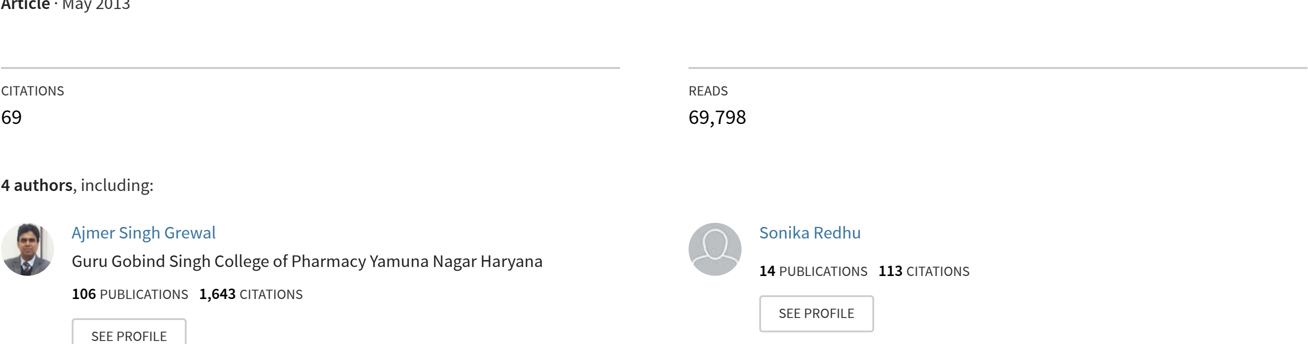

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Review Article

MICROWAVE ASSISTED SYNTHESIS: A GREEN CHEMISTRY APPROACH

Ajmer Singh Grewal, Karunesh Kumar, Sonika Redhu, Shashikant Bhardwaj
Jan Nayak Ch. Devi Lal Memorial College of Pharmacy, Barnala Road, Sirsa- 125055, Haryana, India

Corresponding Author: Ajmer Singh Grewal; Email: ajmergrewal2007@gmail.com

Abstract: Green Chemistry with its twelve principles would like to see changes in the conventional chemical synthesis and the use of less toxic starting materials. Green Chemistry would like to increases the efficiency of synthetic methods, to use less toxic solvents, reduce the stages of the synthetic routes and minimize waste as far as practically possible. In this way, chemical synthesis will be part of the effort for sustainable development. Microwave assisted synthesis has revolutionized chemical synthesis. Small molecules can be built in a fraction of the time required by conventionalmethods. In conventional heating methods oil bath or hot plate are used as a source of heat to a chemical reactionMicrowave irradiation is widely used as a source of heating in chemical synthesis.The basic mechanisms observed in microwave assisted synthesis are dipolar polarization and conduction. Microwave-assisted synthesis provides clean synthesis with the advantage of enhanced reaction rates, higher yields, greater selectivity, and economic for the synthesis of a large number of organic molecules, have provided the momentum for many chemists to switch from conventional heating method to microwave assisted chemistry.Microwave-assisted synthesis is rapidly becoming the method of choice in modern chemical synthesis and drug discovery. The present article will highlight the applications of microwave-assisted synthesis in organic synthesis, inorganic synthesis, polymer synthesis, nanotechnology, peptide synthesis and discuss the basic mechanism involved in microwave heating.

Key words: Microwave heating, Green chemistry, Microwave synthesis, Microwaves.

INTRODUCTION

The term Green Chemistry is becoming the worldwide term used to describe the design of chemical products and processes that reduce or eliminate the use or generation of substances hazardous to human health.[1] The term was coined by the US Environmental Protection Agency and has been defined as: the utilization of a set of principles that reduce or eliminate the use or generation of hazardous substances in the design, manufacture and application of chemical products.[2] This goal can be achieved by use of twelve principles of Green Chemistry which are as follows.

(1) It is better to prevent waste than to treat or clean up waste after it has been created. (2) Synthetic methods should be designed to maximize the incorporation of all materials used in the process, into the final product. (3) Synthetic methods should be designed to use and generate less hazardous/toxic chemicals. (4) Chemical products should be designed to affect their desired function while minimizing their toxicity. (5) The use of solvents and auxiliary substances should be made unnecessary wherever possible and innocuous when used. (6) Energy requirements of chemical processes should be minimized, and synthetic methods should be conducted at ambient temperature and pressure if possible. (7) A raw material should be renewable rather than depleting whenever practicable. (8) Unnecessary derivatization should be minimized or avoided if possible. (9) Catalytic reagents are superior to stoichiometric reagents. (10) Chemical products should be designed so that at the end of their function they break down into innocuous degradation products that do not persist in the environment. (11) Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances. (12) Substances and the form of a substance used in a chemical process should be chosen to minimize the potential for chemical accidents.[2-4]

Organic synthesis on a large scale involves the use of basic chemical ingredients from the petrochemical sector and catalysts; and after the end of the reaction, separation, purification, storage, packaging, distribution etc. Conventional methods of organic synthesis usually need longer heating time, tedious apparatus setup, which result in higher cost of process and the excessive use of solvents/reagents. During these processes there are many problems of health and safety for workers in addition to the environmental problems caused by their use and disposition as waste. Green Chemistry would like to increases the efficiency of synthetic methods, to use less toxic solvents, reduce the stages of the synthetic routes and minimize waste as far as practically possible.[5] Microwave synthesis is considered as an important approach toward green chemistry, because this technique is more eco-friendly. Due to its ability to couple directly with the reaction molecule and by passing thermal conductivity leading to a rapid rise in the temperature, microwave irradiation has been used to improve many organic syntheses.[6,7]

Microwave chemistry is the science of applying microwave radiation to chemical reactions. Microwave synthesis represents a major breakthrough in synthetic chemistry methodology; a dramatic change in the way chemical

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synthesis is performed. Conventional heating, long known to be inefficient and time consuming, has been recognized to be creatively limiting too. Microwave synthesis gives the chemists more time to expand their creativity, test new theories and develop new processes. Instead of spending hours or even days synthesizing a single compound, chemists can now perform the same reaction in minutes. The problem associated with waste disposal of solvents has been overcome by performing reactions without a solvent under microwave irradiation. Coupling of microwave irradiation with the use of mineral-supported catalysed reactions, under solvent-free conditions, provides clean chemical processes with the advantage of enhanced reaction rates, higher yields, greater selectivity, and greater ease of manipulation. Thus microwave synthesis acts as a potential tool for green chemistry.[6, 8]

Microwave irradiation provides an alternative to the conventional methods, for heating or introducing energy into the system. It utilizes the ability of mobile electric charges present in liquid or conducting ions in solid to transform electromagnetic energy into heat. Microwave radiations are electromagnetic waves. In the electromagnetic spectrum, the microwave radiation region is located between infrared radiation and radio waves. Microwaves have wavelength of 1 mm to 1 m corresponding to frequencies between 0.3 and 300 GHz. Telecommunication and microwave radar equipment occupy many of the band frequencies in this region. Microwave dielectric heating; uses the ability of some liquids and solids to transform electromagnetic radiation into heat to drive chemical reactions. This technology opens up new opportunities to the synthetic chemist in the form of new reactions that are not possible using conventional heating.[7,9]

MECHANISM OF MICROWAVE HEATING

All the materials are not susceptible to microwave heating as response of various materials to microwave radiation is diverse. Based on their response to microwaves, materials can be broadly classified as follows:

(1) Materials that are transparent to microwaves, e.g. sulphur
(2) Materials that reflect microwaves, e.g. copper
(3) Materials that absorb microwaves, e.g. water

Microwave absorbing materials are of utmost important for microwave chemistry and three main different mechanisms are involved for their heating namely: Dipolar polarization, Conduction mechanism and Interfacial polarization.[10]

Dipolar polarization:

For a substance to generate heat when irradiated with microwaves it must possess a dipole-moment. It is the electric field component of the microwave radiation, rather than magnetic field component that is responsible for heating, when a dipole tries to reorient itself with respect to an alternating electric field; it loses energy in the form of heat, by molecular friction. Dipolar polarization can generate heat by either interaction between polar solvent molecules such as water, methanol and ethanol; or interaction between polar solute molecules such as ammonia and formic acid. The key requirement for dipolar polarization is that the frequency range of the oscillating field should be appropriate to enable adequate inter-particle interaction. If the frequency range is very high, inter-molecular forces will stop the motion of a polar molecule before it tries to follow the field, resulting in inadequate inter-particle interaction. On the other hand, if the frequency range is low, the polar molecule gets sufficient time to align itself in phase with the field. Microwave radiation has the appropriate frequency (0.3-30 GHz) to oscillate polar particles and enable enough inter-particle interaction. This makes it an ideal choice for heating polar solutions.[11, 12]

Conduction mechanism:

The conduction mechanism generates heat through resistance to an electric current. The oscillating electromagnetic field generates an oscillation of electrons or ions in a conductor, resulting in an electric current. This current faces internal resistance, which heats the conductor. A solution containing ions, or even a single isolated ion with a hydrogen bonded cluster, in the sample the ions will move through the solution under the influence of an electric field, resulting in expenditure of energy due to able to believe that the more polar the solvent, the more readily the microwave irradiation is absorbed and the higher the temperature obtained. Where the irradiated sample is an electrical conductor, the charge carriers (electrons, ions, etc.) are moved through the material under the influence of the electric field, resulting in a polarization. These induced currents will cause heating in the sample due to any electrical resistance. Major limitation of the method is that it is not applicable for materials with high conductivity, since such materials reflect most of the energy that falls on them.[11]

Interfacial polarization:

The interfacial polarization method can be considered as a combination of both the conduction and dipolar polarization mechanisms. It is important for heating systems that comprise a conducting material dispersed in a non-conducting material. For example, consider the dispersion of metal particles in sulphur. Sulphur does not respond to microwaves and metals reflect most of the microwave energy they are exposed to, but combining the two makes them a good microwave-absorbing material. However, for this to take place, metals have to be used in powder form. This is because, unlike a metal surface, metal powder is a good absorber of microwave radiation. It absorbs radiation and is heated by a mechanism that is similar to dipolar polarization. The environment of the metal powder acts as a solvent for polar molecules and restricts the motion of ions by forces that are equivalent to inter-particle interactions in polar solvents. These restricting forces under the effect of an oscillating field induce a phase lag in the motion of ions, resulting in random motion of ions, and ultimately heating of the system.[13-15]

MICROWAVE VERSUS CONVENTIONAL SYNTHESIS

Conventional synthesis usually involves the use of a furnace or oil bath which heats the walls of the reactors by convection or conduction (Figure 1). The core of the sample takes much longer to achieve the target temperature. This is a slow and inefficient method for transferring energy into

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the reacting system. On the other hand in microwave assisted synthesis microwave penetrates inside the material and heat is generated through direct microwave-material interaction (**Figure 1**). Microwave-assisted synthesis has several advantages over conventional reactions in that the microwave allows for an increase in reaction rate, rapid reaction optimization, and rapid analogue synthesis. It also uses both less energy and solvent, and it enables difficult compound synthesis. Specifically, microwave synthesis has the potential to impact upon medicinal chemistry efforts in at least three major phases of the drug discovery process: lead generation, hit-to-lead efforts, and lead optimization. Microwave chemistry can be carried out very efficiently in a parallel format using dedicated rotors or microtiter plate systems. Several hundred reactions can be performed in a single microwave experiment using multimode microwave devices. Researchers have shown the benefits gained by employing microwave heating in tandem with combinatorial chemistry.[16, 17]

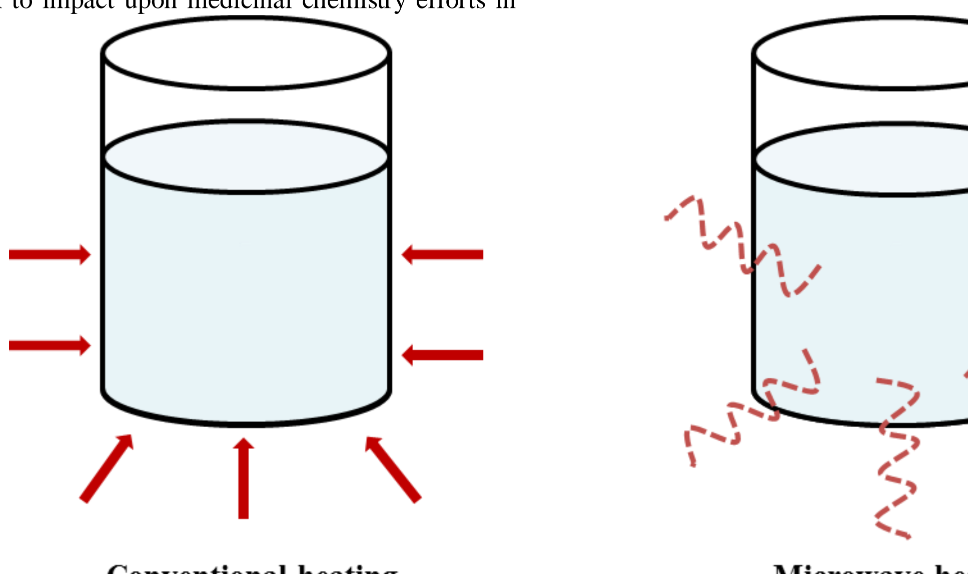

Figure 1: Comparison of microwave heating versus conventional heating[18]

A few reactions which were carried out using microwave heating and compared with conventional heating indicating time and energy efficiency of the technique are compiled in **Table 1**.

Table 1: Comparison of reaction times using microwave versus conventional heating[19]

Compound synthesized	Reaction time: Microwave	Reaction time: Conventional
Methyl benzoate	5 minutes	8 hours
4-nitrobenzyl ester	2 minutes	1.5 hours
Zeolite synthesis	30 seconds	60 minutes
Cubanite	3 minutes	3 days
$NaAlH_4$	2 hours	8 hours
$CuBi_2O_4$	5 minutes	18 hours
Ag_3In	2 minutes	48 hours

MICROWAVE SYNTHESIS APPARATUS

The apparatus for microwave assisted synthesis include; single-mode microwave ovens, and multi-mode microwave ovens.[9]

Single-mode microwave apparatus:

The differentiating feature of a single-mode apparatus is its ability to create a standing wave pattern. This interface generates an array of nodes where microwave energy intensity is zero, and an array of antinodes where the magnitude of microwave energy is at its highest. One of the limitations of single-mode apparatus is that only one vessel can be irradiated at a time. However, the apparatus is user-friendly. An advantage of single-mode apparatus is their high rate of heating. This is because the sample is always placed at the antinodes of the field, where the intensity of microwave radiation is the highest. These apparatus can process volumes ranging from 0.2 to about 50 ml under sealed-vessel conditions, and volumes around 150 ml under open-vessel conditions.Single-mode microwave ovens are currently used for small-scale drug discovery, automation and combinatorial chemical applications.

Multi-mode microwave apparatus:

An essential feature of a multi-mode apparatus is the deliberate avoidance of generating a standing wave pattern inside it. The goal is to generate as much chaos as possible inside the apparatus. The greater the chaos, the higher is the dispersion of radiation, which increases the area that can cause effective heating inside the apparatus. As a result, a multi-mode microwave heating apparatus can accommodate

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a number of samples simultaneously for heating, unlike single-mode apparatus where only one sample can be irradiated at a time. Owing to this characteristic, a multi-mode heating apparatus is used for bulk heating and carrying out chemical analysis processes such as ashing, extraction, etc.In large multi-mode apparatus, several litres of reaction mixture can be processed in both open and closed-vessel conditions. A major limitation of multi-mode apparatus is that, heating samples cannot be controlled efficiently because of lack of temperature uniformity.[20-24]

BENEFITS OF MICROWAVE ASSISTED SYNTHESIS

Microwaves can accelerate the rate of reaction, provide better yields and higher purity, uniform and selective heating with lower energy usage, achieve greater reproducibility of reactions and help in developing convenient and cleaner synthetic routes. The main advantages of microwave assisted organic synthesis are:

Faster reaction: Based on experimental data it has been found that microwave-enhanced chemical reaction rates can be faster than those of conventional heating methods by as much as 1,000-fold. The microwave can use higher temperatures than conventional heating system, and consequently the reactions are completed in few minutes instead of hours, for instance, synthesis of fluorescein, which usually takes about 10 hours by conventional heating methods, can be conducted in only 35 minutes by means of microwave heating.

Better yield and higher purity: Less formation of side products are observed using microwave irradiation, and the product is recovered in higher yield. Consequently, also the purification step is faster and easier. For example, microwave synthesis of aspirin results in an increase in the yield of the reaction, from 85 % to 97 %.

Energy saving: Heating by means of microwave radiation is a highly efficient process and results in significant energy saving. This is primarily because microwaves heat up just the sample and not the apparatus, and therefore energy consumption is less.

Uniform and selective heating: In conventional heating, the walls of the oil bath get heated first, and then the solvent. As a result of this distributed heating in an oil bath, there is always a temperature difference between the walls and the solvent. In the case of microwave heating, only the solvent and the solute particles are excited, which results in uniform heating of the solvent. Selective heating is based on the principle that different materials respond differently to microwaves. Some materials are transparent whereas others absorb microwaves.

Green synthesis: Reactions conducted using microwaves are cleaner and more eco-friendly than conventional heating methods. Microwaves heat the compounds directly; therefore, usage of solvents in the chemical reaction can be reduced or eliminated. Synthesis without solvent, in which reagents are absorbed on mineral support, has a great potential as it offers an eco-friendly green protocol in synthesis. The use of microwaves has also reduced the amount of purification required for the end products of chemical reactions involving toxic-reagents.

Reproducibility: Reactions with microwave heating are more reproducible compared to the conventional heating because of uniform heating and better control of process parameters. The temperature of chemical reactions can also be easily monitored.[25-31]

LIMITATIONS OF MICROWAVE ASSISTED SYNTHESIS

The yield obtained by using microwave apparatus available in the market is limited to a few grams. Although there have been developments in the recent past, relating to the scalability15 of microwave equipment, there is still a gap that needs to be spanned to make the technology scalable. The use of microwaves as a source of heating has limited applicability for materials that absorb them. Microwaves cannot heat materials such as sulphur, which are transparent to their radiation. Improper use of microwave heating for rate enhancement of chemical reactions involving radioisotopes may result in uncontrolled radioactive decay. Certain problems, with dangerous end results, have also been observed while conducting polar acid-based reactions, for example, microwave irradiation of are action involving concentrated sulphuric acid may damage the polymer vessel used for heating. Conducting microwave reactions at high-pressure conditions may also result in uncontrolled reactions and cause explosions. Health hazards related to microwaves are caused by the penetration of microwaves. While microwaves operating at a low frequency range are only able to penetrate the human skin, higher frequency-range microwaves can reach body organs. Research has proven that on prolonged exposure microwaves may result in the complete degeneration of body tissues and cells. It has also been established that constant exposure of DNA to high-frequency microwaves during a biochemical reaction may result in complete degeneration of the DNA strand.[19, 32, 33]

ENHANCED MICROWAVE SYNTHESIS

Recently, an alternative method for performing microwave-assisted organic reactions, termed Enhanced Microwave Synthesis (EMS), has been examined. By externally cooling the reaction vessel with compressed air, while simultaneously administering microwave irradiation, more energy can be directly applied to the reaction mixture. EMS ensures that a high, constant level of microwave energy is applied. Simultaneous cooling enables a greater amount of microwave energy to be introduced into a reaction, while keeping the reaction temperature low. This results in significantly greater yields and cleaner chemistries. EMS was employed in the synthesis of a variety of α-keto amides (Scheme 1) to support a protease inhibitor discovery project. This may eventually lead to improved treatments for stroke, Alzheimer's disease, and muscular dystrophy. Under conventional heating conditions, this took between 2 to 6 hours for completion; whereas under optimized EMS conditions, the two steps were completed in 2 min and in 21-74% yields.[34, 35]

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$R^1C(O)Cl + NC-R^2 \xrightarrow[\text{simultaneous cooling}]{\text{neat, microwave 150 W, 1 minute}} [R^1C(O)C(Cl)=N-R^2] \xrightarrow[\text{simultaneous cooling}]{CaCO_3,\ H_2O,\ \text{microwave 100 W, 1 minute}} R^1C(O)C(O)NH-R^2$

Scheme 1: Improved Synthesis of α-keto Amides by Enhanced Microwave Synthesis

APPLICATIONS OF MICROWAVE ASSISTED SYNTHESIS

Application of microwave irradiation in chemical synthesis involves its use in the acceleration of chemical synthesis. Microwave-enhanced synthesis results in faster reactions, higher yields, and increased product purity. In addition, due to the availability of high-capacity microwave apparatus, the yields of the experiments have now easily scaled up from milligrams to kilograms, without the need to alter reaction parameters. Microwave-assisted synthesis can be suitably applied to the drug discovery process.[33]

Organic synthesis:

Microwave-assisted organic synthesis has been the foremost and one of the most researched applications of microwaves in chemical reactions. Literature survey reveals that scientists have successfully conducted a large range of organic reactions. These include Diels-Alder reaction, Ene reaction, Heck reaction, Suzuki reaction, Mannich reaction, Hydrogenation of [beta]-lactams, Hydrolysis, Dehydration, Esterification, Cycloaddition reaction, Epoxidation, Reductions, Condensations, Cyclisation reactions, Protection and deprotection, etc.[34]

Microwave-assisted organic synthesis is being widely applied in the pharmaceuticals industry, particularly for developing compounds in the lead optimization phase of drug discovery and development. In this phase, chemists use diverse synthetic techniques to develop candidate drugs from lead compounds. Based on reaction conditions, organic synthesis reactions can be conducted in the following techniques.

(1) **Microwave-assisted organic synthesis at atmospheric pressure:** Microwave-assisted organic synthesis can be most conveniently conducted at atmospheric pressure in reflux conditions, for example, oxidation of toluene to benzoic acid (**Scheme 2**) with $KMnO_4$ under normal conditions of refluxing takes 10-12 hours compared to reaction in microwave conditions, which takes only 5 minutes. **Table 2** shows an increased yield of 200 % for the oxidation of hexanenitrile and 150 % for the hydrolysis of cyclohexene when the reaction is conducted in the microwave batch reactor.[36, 37]

Toluene $\xrightarrow[\text{aq. } KMnO_4,\ \text{aq. KOH, MW 5 minutes}]{[O]}$ Benzoic acid (COOH)

Scheme 2: Oxidation of toluene to benzoic acid with $KMnO_4$

Table 2: Heterogeneous reactions under microwave and classical heating[36]

Chemical reaction	Time (minutes)	MW Yield (%)	Classical Yield (%)
Hydrolysis of hexanenitrile	60	40	26
Oxidation of cyclohexene	60	26	12

(2) **Microwave-assisted organic synthesis at elevated pressure:** Microwaves can be used to directly heat the solvents in sealed microwave-transparent containers. The sealed container helps in increasing the pressure in the reactor, which facilitates the reaction that will take place at much higher temperatures. This results in a substantial increase in the reaction rate of microwave-assisted organic synthesis.[12]

(3) **Microwave-assisted organic synthesis under solvent-free conditions:** Microwave-assisted solvent-free organic synthesis has been developed as an environmentally friendly process as it combines the selectivity associated with most reactions carried out under microwaves with solvent and waste-free procedures in which organic solvents are avoided throughout all stages. The solvent-free organic syntheses are of three types: (i) reactions using neat reactants; (ii) reactions using solid-liquid phase transfer catalysis (PTC); and (iii) reactions using solid mineral supports. The microwave-assisted reaction could be completed within two to three minutes, compared to conventional oil-bath heating at 75 °C for 40 hours.[12, 38]

Inorganic synthesis:

A variety of materials such as carbides, nitrides, complex oxides, silicides, zeolites, apatite, etc. have been synthesized using microwaves. A series of A_3B and A_4 type mesoporphyrinic complexes were synthesized with superior yields using microwave irradiation under solvent-free conditions. Solvent-free synthesis by microwave irradiation has been successfully applied to obtaining mesoporphyrinic compounds because the absence of solvent from the reaction environment has the effect of decreased interaction time between reactant molecules and improves the reaction yield. Two new iso-structural coordination polymers with novel anionic metal-organic frameworks were synthesized using

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microwave-assisted technique. Microwave-assisted synthesis of pinacol boronates from aryl chlorides catalysed by a palladium/imidazolium salt system was reported.[39-43]

Synthesis of nanotechnology products:
Amongst the several methods that exist for synthesizing of nanoparticles, the use of microwave assisted synthesis has shown promise. Synthesis of silver nanoparticles from silver nitrate employing starch as the reductant as well as stabilizing agent has been carried out under direct heating, controlled heating and microwave irradiation. The microwave irradiation was considered as better for reduction of silver ions to silver nanoparticles. It also afforded smaller particle sizes and particle size distribution. Compared to conventional methods, microwave assisted synthesis was faster and provided particles with an average particle size of 12 nm. Nanostructures with smaller sizes, narrower size distributions, and a higher degree of crystallization were obtained under microwave heating than those in conventional oil-bath heating. The gold nanoparticles have been prepared by microwave high-pressure procedure with alcohol as the reducing agent. A method has been reported for microwave-assisted non-aqueous synthesis of zinc oxide nanoparticles. Particularly the fast reaction rates, better product yields and the possibility to automatically combine different experimental parameters makes microwave-assisted synthesis suitable for the studies of the influences of the reaction conditions on the morphology and sizes of zinc oxide nanoparticles particles, which determine its properties and applications. Pt/C and $PtCo_3O_4/C$ nano catalysts were prepared using microwave assisted methods. The results of XRD and TEM revealed that the prepared catalysts have small and uniform shapes with high dispersion ability. The developed approach is a useful method for preparing platinum and platinum supported electrocatalysts, which can be used in the field of fuel cells and other related fields. Strontium stannate ($SrSnO_3$) nanostructures were obtained by microwave-assisted calcination of a $SrSn(OH)_6$ precursor powder. Compared to other conventional calcination methods mentioned in the literature, this procedure led to a remarkable decrease of the reaction time and the synthesis temperature owing to direct interaction of radiation with the material.[9, 44-50]

Polymer synthesis:
Polymer chemistry, including ceramic processing, forms the single-largest application area of microwave chemistry. The use of polar reactants in polymerization reaction results in controlled synthesis and a combination of this with direct heating of reactants makes microwave heating an economically viable option. Using microwave radiation in curing has greatly increased the rate of the reactions. It has been found that the rate of a curing reaction, using microwaves, is not dependent on the power applied but on the way the pulse is applied. Controlled solvent-free synthesis and modification in polymer materials can be rapidly and effectively done with the help of microwave heating using large scale reactors The first microwave assisted organic synthesis of Poly Lactic Acid was carried out with SnOct as catalyst by using toluene as a solvent.[51, 52]

Peptide synthesis:
A microwave-assisted, rapid solid phase peptide synthesis procedure has been reported. The synthesis protocol is based on the use of cycles of pulsed microwave irradiation with intermittent cooling of the reaction during the removal of the Fmoc protecting group and during the coupling. The desired nonapeptide was obtained in highest yield and purity by employing MicroKan technology. The protocols for the synthesis of cystine-rich peptides in the presence of microwave radiation with solid phase peptide synthesis have been reported. The method is broadly applicable for a wide range of peptides using Boc-SPPS, especially for SPPS of large peptides via native chemical ligation. Microwave radiation produces peptides in high yield and with high purity, and the time for the assembly of approximately 30 amino acids peptide chains was reduced to an overnight reaction in the automated microwave-assisted synthesis. The applications of microwaves in the field of peptides and glycopeptides have been reported.[53-56]

Synthesis of radiopharmaceuticals:
Microwave-assisted organic synthesis at an elevated pressure has been used in pharmaceutical industry for the synthesis of radiopharmaceuticals. During pre-clinical trials, these radiopharmaceuticals are used as tracers to generate a nuclear medical image. A multi-mode microwave oven was used in the first trial of this kind and it was observed that the rate of reaction increased substantially. This has resulted in the enhanced use of microwaves to produce radiopharmaceuticals. Advantages of microwaves include the fast reaction rates and high yield of the reaction. This can be attributed to the short half-life of reactants, for example, saving five minutes in a synthesis with carbon-11 resulted in an enhanced production rate of 15%. It has also been observed that several reactions could only be achieved by using microwaves.[57-60]

CONCLUSION
Microwave-assisted synthesis is a convenient way toward the goal of green chemistry.Microwaves irradiation can be used to in chemical synthesis as a heat source; it is veryefficient and can be used to significantly reduce reaction times of numerous synthetically useful chemical transformations.Thus, microwave-assisted synthesis has advantages over conventional technology: it is more energy efficient and it can lead to improved isolated yields of products with green synthesis.The advantages of this enabling technology have, more recently, been exploited in the context of multistep total synthesis and medicinal chemistry/drug discovery, and have additionally penetrated related fields such as polymer synthesis, material sciences, nanotechnology and biochemical processes. In order to achieve further development inthis field, novel instruments, which give rise to reproducible performances and that constitute aminimal hazard should be used instead of the domestic microwave ovens.

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REVIEW ARTICLE

Quality by Design (QbD) concept Review in Pharmaceuticals

Kaustubh Jagtap*, Bharati Chaudhari, Vivekkumar Redasani
YSPM'S, Yashoda Technical Campus, Faculty Of Pharmacy, Wadhe, Satara.
*Corresponding Author E-mail: **kaustubhjagtap121@gmail.com**

ABSTRACT:
Quality by Design (QbD) refers to a holistic approach towards drug development. Quality by design is a vital part of the modern approach to pharmaceutical quality. The purpose of this practice school topic is to discuss the pharmaceutical Quality by Design (QbD) and illustrate how it can be used to ensure pharmaceutical quality. The QbD is a systemic approach to pharmaceutical development. It means designing and developing formulations and manufacturing processes to ensure predefined product quality. Some of the QbD elements include: Defining Quality target product profile, identifying critical quality attributes, link the drug excipients attributes, establishing design space, control strategy, critical process parameters and product life cycle management. Using QbD, pharmaceutical quality is assured by understanding and controlling formulation and manufacturing variables. A new approach to drug development could increase efficiencies, provide regulatory support and flexibility, and offer important business benefits throughout the product's life cycle. This PS topic explores the processes used in developing a market formulation and required supportive data, particularly in light of the industry's current movement toward submissions based on QbD. The work also facilitates the adoption and implementation of QbD. Principles in the development of pharmaceutical industries. Successful implementation of QbD concepts requires cooperation across a multitude of company teams, from R&D to manufacturing to quality control and regulatory affairs. This is necessary to ensure that QbD concepts are incorporated not only when the first activities are initiated around a product's design but also during the design of the process used to make the product and other activities associated with a product's life cycle. The application of the concept of quality by design (QbD) presented in this paper aligns with the principles of ICH Q8, Q9 and Q10 guidelines.

KEYWORDS: Control strategy, Critical material attributes, Critical process parameters, Design space, Quality by design.

INTRODUCTION:

Quality by Design (QbD) was first described by Joseph M. Juran. and applied heavily, particularly in the automotive industry. The fundamental premise behind QbD is that quality can be "designed in" to processes through systematic implementation of an optimization strategy to establish a thorough understanding of the response of the system quality to given variables, and the use of control strategies to continuously ensure quality.

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The FDA has recently begun to advocate the QbD methodology for the pharmaceutical sector. In order to describe quality by design, we must first define what we mean by quality. In a 2004 paper, Janet Woodcock (Director for the Centre for Drug Evaluation and Research) defined pharmaceutical quality as a 'product that is free of contamination and reproducibly delivers the therapeutic benefit promised in the label to the consumer'. This explanation focuses on the QbD for generic drugs. The concept of QbD was mentioned in the ICH Q8 guidance, which states that "quality cannot be tested into products, i.e., quality should be built in by design". This paper discusses the pharmaceutical quality by design and describes how it can be used to ensure pharmaceutical quality with emphasis on solid oral dosage forms of small molecules. The pharmaceutical industry works hard to develop, manufacture, and bring

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to market new drugs and to comply with regulatory requirements to demonstrate that the drugs are safe and effective. A new approach to drug development could increase efficiencies, provide regulatory relief and flexibility, and offer important business benefits throughout the product's life cycle. This topic explores the processes used in developing a market formulation and requisite supportive data, particularly in light of the industry's current QbD concept as part of its two-year initiative, movement toward submissions based on quality by design (QbD). DA introduced the Pharmaceutical cGMPs for the 21st Century: A Risk-Based Approach Pharmaceutical cGMP initiative (also referred to as the pharmaceutical cGMP Initiative or 21st Century Initiative) in 2002. QbD is not a new concept from a pharmaceutical technology perspective. It is, however, a new concept relative to pharmaceutical regulatory review and submission. As a systematic and prospective approach to product design, process design and control, process performance and continuous improvement, QbD designs quality into the manufacturing process. By doing so, QbD encourages innovation, continuous quality improvement, and science-and risk based regulatory processes and ensures the availability of high-quality medicines to the consumer.[1,2,3,4]

Design:
Product is designed to meet patient needs and performance requirements. Process is designed to consistently meet product quality attributes. Impact of starting raw materials and process parameters on product quality is understood. Critical sources of process variability are identified and controlled. The process is continually monitored and updated to allow for consistent quality over time.[5]

Quality:
"The degree to which a set of inherent properties of a product, system or process fulfils requirements" (ICH Q9).

"Good pharmaceutical quality represents an acceptably low risk of failing to achieve the desired clinical attributes."[1]

Definition of QbD [ICH Q8 (R1)]
A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management.[5]

Definition of PAT [FDA PAT Guidelines, Sept. 2004]
A system for designing, analysing and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of new and in-process materials and processes, with the goal of ensuring final product safety.[5]

Process Analytical Technology
The concept actually aims at understanding the processes by defining their Critical process parameters, and accordingly monitoring them in a timely manner (preferably in-line or on-line) and thus being more efficient in testing while at the same time reducing over-processing, enhancing consistency and minimizing rejects.

The FDA has outlined a regulatory framework for PAT implementation. With this framework–according to Hinze "the FDA tries to motivate the pharmaceutical industry to improve the production process". Because of the tight regulatory requirements and the long development time for a new drug, the production technology is "frozen" at the time of conducting phase-2 clinical trials.

PAT allows for and encourages continuous process manufacturing improvement. It uses real-time information to reduce process variation and manufacturing capability and demands a solid understanding of the various processes involved in the operation. Simply put PAT is a real-time testing and adjustment based on the complete understanding of how the components and related processes affect the final product. This is in accordance with the fundamental principle that quality cannot be tested but is instead built into the medicinal product by design.[6]

PAT is a system for

- Designing, analysing and controlling manufacturing.
- Timely measurements.
- Critical quality and performance attribute.
- Raw and in-process materials.
- And processes.[6]

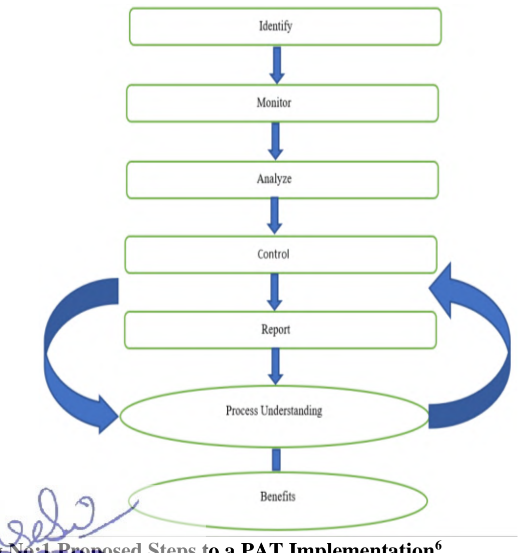

Fig.No.1 Proposed Steps to a PAT Implementation[6]

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Benefits of QbD:

- QbD is good Business.
- Eliminate batch failures.
- Minimize deviations and costly investigations.
- Avoid regulatory compliance problems.
- Organizational learning is an investment in the future.
- QbD is good Science.
- Better development decisions.
- Empowerment technical of staff.[7,8,9]

Opportunities of QbD:

- Efficient, agile, flexible system.
- Increase manufacturing efficiency, reduce costs and project rejections and waste.
- Build scientific knowledge base for all products.
- Better interact with industry on science issues.
- Ensure consistent information.
- Incorporate risk management.[6,8]

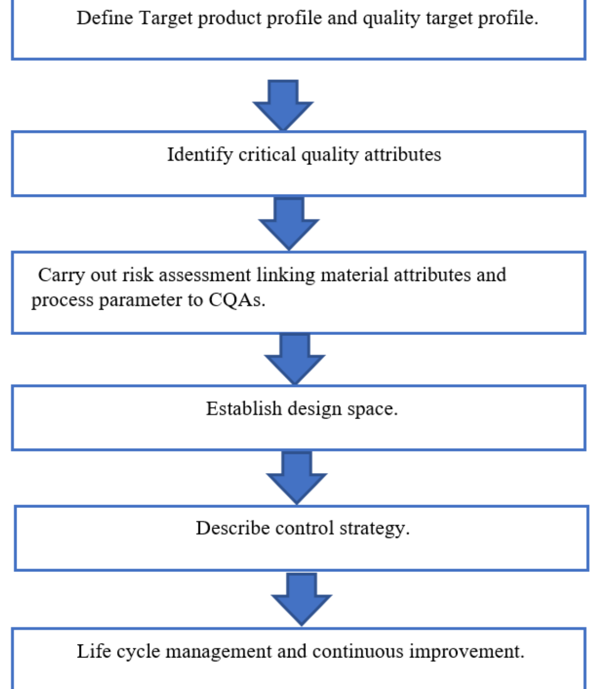

Fig.No:2 Flow of Quality by Design[1]

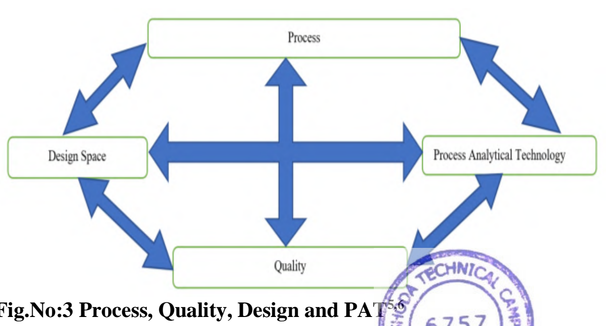

Fig.No:3 Process, Quality, Design and PAT[5,6]

Steps of Quality by Design:

1. Target Product Profile (TPP):

FDA published a recent guidance defining a Target Product Profile (TPP): "The TPP provides a statement of the overall intent of the drug development program, and gives information about the drug at a particular time in development. Usually, the TPP is organized according to the key sections in the drug labelling and links drug development activities to specific concepts intended for inclusion in the drug labelling." When ICH Q8 says that pharmaceutical development should include "...identification of those attributes that are critical to the quality of the drug product, taking into consideration intended usage and route of administration", the consideration of the intended usage and route of administration would be through the TPP.

Identifying Quality Target Product Profile (Qtpp):

"Begin with the end in mind" By Beginning with the end in the mind, the result of development is robust formulation and manufacturing process with an acceptable control strategy that ensures the performance of the drug product. The quality target product profile (QTPP) is "a prospective summary of the quality characteristics of a drug product that ideally will be achieved to ensure the desired quality, taking into account safety and efficacy of the drug product." The QTPP is an essential element of a QbD approach and forms the basis of design of the generic product. The quality target product profile (QTPP) is a quantitative substitute for aspects of clinical safety and efficacy.[1,2,5,13]

Quality target product profile (QTPP) Includes, but not limited to:

- Dosage form.
- Route of administration.
- Strength.
- Release or Delivery of the drug.
- Pharmacokinetic characteristics e.g., dissolution, aerodynamic performance.
- Drug product quality characteristics for intended use e.g., sterility, purity.[1,2,5]

2. Identifying Critical Quality Attributes (CQA):

Definition: ICH Q8 (R1) defines CQAs as physical, chemical, biological or microbiological properties or characteristics that should be within an appropriate limit, range, or distribution to ensure the desired product quality. The International Society of Pharmaceutical Engineers (ISPE) and Product Quality Lifecycle Implementation (PQLI) defines critical quality attributes (CQAs) as physical, chemical, biological or microbiological properties or characteristics that need to be controlled (directly or indirectly) to ensure product quality. CQA has been used by some to describe elements of the QTPP (such as dissolution) while others have used CQA to describe mechanistic factors (such as particle size and hardness) that determine product

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performance. Thus, CQA is used to describe both aspects of product performance and determinants of product performance. It was stated that the ICH working definition of CQA was: “A CQA is a quality attribute (a physical, chemical, biological or microbiological property or characteristic) that must be controlled (directly or indirectly) to ensure the product meets its intended safety, efficacy, stability and performance”. This CQA definition implies that the intended safety, efficacy, stability and performance are not CQAs. Safety and efficacy clearly fall under the domain of the TPP But if stability and performance are not CQA and not part of the TPP, then what are they? We are thus compelled to acknowledge that there is an intermediate category of product performance (or surrogates for quality) that we have defined as the QTPP.[1,2]

3. Critical Process Parameter:

Critical process parameter (CPP) is defined as any measurable input (input material attribute or operating parameter) or output (process state variable or output material attribute) of a process step that must be controlled to achieve the desired product quality and process uniformity. In this view, every item would be a process parameter. Surrogates for quality) that we have defined as the QTPP.

For a given unit operation, there are four categories of parameters and attributes:

- Input material attributes
- Output material attributes
- Input operating parameters
- Output process state conditions.

Critical Process Parameter:

A parameter is Critical when a realistic change in that parameter can cause the product to fail to meet the QTPP. Uniqueness of Critical Process Parameters: Because of the broadness of the CPP definition it is possible for two investigators to examine the same process and come to a different set of CPP. The set of CPP is not unique, but the chosen set must be sufficient to ensure product quality. Different sets of CPP can have several origins. One is that the definition of operating parameters depends on the engineering systems installed on a piece of process equipment.[1,2,10,12]

4. Risk Assessment and Design Space:

Quality Risk Management (ICH Q9) indicates that, the manufacturing and use of a drug product necessarily entail some degree of risk. Risk assessment is a valuable science-based process used in science-quality risk management that can aid in identifying which material attributes and process parameters potentially have an effect on product CQAs. Risk assessment is typically performed early in the pharmaceutical development process and is repeated as more information becomes available and greater knowledge is obtained. Risk assessment tools can be used to identify and rank parameters (e.g., process, equipment, input materials) with potential to have an impact on product quality, based on prior knowledge and initial experimental data.

Design space ICH Q8 (R1) defines Design space as, the multidimensional combination and interaction of input variables (e.g., material attributes) and process parameters that have been demonstrated to provide assurance of quality. Working within the design space is not considered as a change. Movement out of the design space is considered to be a change and would normally initiate a regulatory post-approval change process. Many believe design space and QbD are interchangeable terms. This is incorrect. For generic-drug applications, design space is optional. QbD can be implemented without a design space because product and process understanding can be established without a formal design space. It should be pointed out that implementation of QbD is strongly encouraged by FDA. For some complex drug substances or drug products, implementation of QbD is considered a required component of the application. Submission of a design space to FDA is a pathway obtaining the ability to operate within that design space without further regulatory approval.[1,2,14]

5. Defining Control Strategy ICH Q8 (R1):

It defines control strategy as A planned set of controls, derived from current product and process understanding that ensures process performance and product quality.

Minimal and enhanced approaches As in ICH Q8(R), a distinction may be drawn between a minimal and an enhanced control strategy approach. In a Minimal Control Strategy, drug product quality is controlled primarily by intermediate (in process material) and end product testing. In an Enhanced Control Strategy drug product quality ensured by risk-based control strategy for well understood product and process, and quality controls are shifted upstream, with the possibility of real-time release or reduced end-product testing.

Developing the Control Strategy:

Development of a Control Strategy requires a structured process, involving a multi-disciplinary team of experts, linking pharmaceutical development to the manufacturing process, and engineering controls of process equipment.

The PQLI Control Strategy Team has proposed a Control Strategy Model that facilitates understanding and that may be used a cross-functional communication tool.

Personnel at all levels should be able to understand the way control strategy links from CQAs to operational aspects to ensure, for example that:

- Chemists understand in-process controls are established to keep the process inside the design

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space and seek opportunities for simplification of controls, as knowledge is gained.

- Engineers know how equipment operating conditions impact product quality.
- Quality Assurance professionals know where the highest risks are in the process.[1,5]

6. Control Strategy and the Product Lifecycle:
The Control Strategy is related to the level of process understanding at a given time, and evolves as manufacturing experience increases. The originally specified measures, controls or models may be modified or even removed, or the need for additional controls may be identified. Other revisions to the Control Strategy may relate to continual improvement, for example the introduction of improved analyser or control technology. Periodic reviews of risk assessments and mitigation should be conducted to determine the appropriateness of the Control Strategy based on product manufacturing history. Failure or deviations should be investigated and the effectiveness of the control system considered in relation to the identified root cause. Corrective and preventive actions should be applied and the Control Strategy updated as necessary (including any regulatory actions required) in the light of new product and process knowledge. Implementing PAT in the Control Strategy will require the application of process models (multivariate prediction models) that either predicts CQAs or CPPs or a combination of both. These models may require frequent updates, depending on the maturity of the model (e.g., the amount of data and their variability within the model), as well as the kind of data that has been included to reflect variability in scale, equipment, analytical set-up, sampling, and site. A monitoring program for verifying the validity of process models should be established and be based on a risk analysis of the model itself and include possible ways to verify the model by other means. One example would be to compare the predicted CQA value to a conventional analytical method. The monitoring program should include requirements for when a model has to be updated (e.g., change of raw material supplier or deviations resulting in increased knowledge).[1,2,11]

CONTINUOUS IMPROVEMENT:

"Continuous improvement is an essential element in a modern quality system that aims at improving efficiency by optimizing a process and eliminating wasted efforts in production. These efforts are primarily directed towards reducing variability in process and product quality characteristics."

The backbone for Continuous Improvement is the Pharmaceutical Quality System. PQS should facilitate continual improvement and help to: "Identify and implement appropriate product quality improvements, process improvements, variability reduction, innovations and pharmaceutical quality system enhancements, thereby increasing the ability to fulfil quality needs consistently. Quality risk management can be useful for identifying and prioritizing areas for continual improvement. "Continuous improvement is not the same as corrective actions preventative actions (CAPA).[1,11,15]

CONCLUSION:

The goal of a well-characterized method development effort is to develop a reliable method that can be implemented with a high degree of assurance to consistently produce data meeting predefined criteria when operated within defined boundaries. QbD can be applied to the development and evaluation of analytical methods. QbD gives an idea about the process development with very detailed analysis of every single part involved in it that can maintain products quality at extreme level. Quality by Design's steps have accurate understanding of product and process development that can avoid unnecessary variables and problems in manufacturing of product that can evaluate and keep consistency in quality of product.

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Yashoda Technical Campus
Satara

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FORMULATION AND EVALUATION OF ASCORBIC ACID EFFERVESCENT GRANULES

Pratiksha S. Londhe*, Poonam D. Khalate, Bharatee P. Chaudhari, Vivekkumar K. Redasani

YSPM, Yashoda Technical Campus Faculty of Pharmacy, Wadhe Satara.

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***Corresponding Author**

Pratiksha S. Londhe

YSPM, Yashoda Technical Campus Faculty of Pharmacy, Wadhe Satara.

ABSTRACT

Effervescent granules have occupied a unique place in the field of pharmaceutics. Widely use in clinical diagnosis of heart burn, urinary tract infection, acidity. The aim of these study to design and optimize effervescent granules of ascorbic acid. Eight different formulation of ascorbic acid effervescent granules was prepared and formulations are made up of chemical ingredients such as citric acid, tartaric acid, sodium bicarbonate and calcium carbonate. Effervescent granules were prepared by heat or fusion method. The study also focused on the water of crystallization concept which is related to the reaction between citric acid, tartaric acid and sodium bicarbonate. Evaluation test were performed such as disintegration test, amount of carbon dioxide, pH of formulation. There were eight different formulation prepared and denoted as F1, F2, F3, F4, F5, F6, F7 and F8. Fifth formulation that is F5 gives precise result. They give significantly low disintegration time, pH within range and also amount of carbon dioxide in accepted range. F5 give significant result as compared to other formulations.

KEYWORDS:- Effervescent granules, Disintegration time, Damped mass, Heat method, Water of crystallization, Ascorbic acid.

INTRODUCTION

Effervescence is Latin word it means escape of gas from an aqueous solution. Effervescent granules have short half-life as react rapidly with polar solvent or water. There is a liberation of carbon dioxide gas due to chemical reaction between acid and base.[1]

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Effervescent granules are one of the solid dosages forms that is taken orally. In daily life, use of effervescent preparation is increasingly due to many advantages such as good stability, quickly dissolve, masking of unpleasant taste and ease administration with highly compliance in patients with difficulty in swallowing of pills and tablet. The bioavailability of low absorbed drugs can be increased by effervescent granules preparation.[2]

Effervescent granules are spherical in shape and very coarse in nature. They were prepared by hot method. Effervescent granules are not administered directly. They are intended to be dispersed in water before use. Effervescent granules are a type of compound powder. For dissolution of effervescent granules only water is used due to acid dissociate in the water and produce hydrogen ions which is needed for evolution carbon dioxide gas.[3]

Due to the high content of carbonate salt, upon the ingestion of drug solution, the gastric pH is temporarily elevated, resulting in first gastric emptying. This in turn promotes drug adsorption from the upper small intestine, which is primary site of drug absorption. Effervescent granules are responsible for higher bioavailability and fast disintegration rates. Within a couple of minutes, the granules are completely dissolved and the drug become available in solution.[4]

The ideal disintegration time for effervescent granules is 6 to 9 sec. While disintegration time for uncoated tablet, coated tablet, film coated tablet and enteric coated tablet is 15min, 60min, 30min and 60min respectively.[5]

Ascorbic acid or vitamin C is water soluble vitamin. They widely used in prevention of scurvy disease. They also prevent oxidation of molecules inside a body. So, they act as potent antioxidant agent. Vitamin C naturally found in citrus fruit, lemon, oranges. Ascorbic acid involved in production of collagen fibres. Also play a vital role in diagnosis of cancer. Vitamin C is potent anti-inflammatory, antibacterial, immunostimulant agent. It is potent antioxidant and cofactor of gene regulating enzymes. Ascorbic acid enhanced the action of B cells and T cells.[6]

The scientist research on the deficiency of vitamin C cause major disease known as scurvy. Ascorbic acid is essential part of diet. Bruising, bleeding gums, weakness, fatigue and rash are among scurvy symptoms. Minimum intake of ascorbic acid causes haemodynamic instability.[7]

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Concept of water crystallization

Crystals of some compound seem to be dry or anhydrous but actually contain some amount of water molecule attached to them. This water molecule is called water of crystallization. Fixed number of water molecules present in formula unit of salt. Hydrated salt contain water in its structure that cause crystallization.

When all excipients and active drug mixed together in a clean porcelain dish. As the heat were provided to the porcelain dish and powder mixture get converted into damp mass or lumps due to water of crystallization. When heat is provided water molecules is released out and water of crystallization is taken place.

Citric acid is reacted with sodium bicarbonate is gives sodium citrate, carbon dioxide, water molecules are release

C6H8O7 + 3NaHCO3 ⟶ C6H5Na3O + 3CO + 3H2O

Tartaric acid is reacted with sodium bicarbonate it gives sodium tartrate, carbon dioxide, water molecule is release.

C4H6O6 + 2NaHCO3 ⟶ C4H5Na3O + 3CO + 3H2[8]

MATERIALS AND METHODS

Chemical used for this formulation are purchased from SD Lab of Mumbai. The excipients were used for preparation such as- Sodium bicarbonate (S D LAB CHEM MUMBAI), Ascorbic acid (S D LAB CHEM MUMBAI), Calcium bicarbonate (S D LAB MUMBAI), Citric acid (S D LAB MUMABAI), Tartaric acid (S D LALB MUMBAI)

Instruments – Hot Plate, Weighing Balance

Glassware – Volumetric flask, stirrer, beaker

Preparation of effervescent granules

There were six formulations of effervescent granules are prepared. Formulation batches are denoted by symbol F.

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Ascorbic acid	1gm	1gm	1gm	1gm	1gm	1gm	1gm	1gm
Citric acid	1gm	0.1gm	1gm	0.1gm	0.1gm	1gm	1gm	0.1gm
Tartaric acid	0.1gm	1gm	1gm	0.1gm	0.1gm	1gm	0.1gm	1gm
Sodium bicarbonate	1.5gm	1.5gm	1gm	1.5gm	1gm	1.5gm	1gm	1gm
Calcium Carbonate	0.203 gm	0.203 gm	0.203 gm	0.203 gm	0.203 gm	0.203 gm	0.203 gm	0.203 gm

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Heat method or fusion method

All excipients were accurately weighted and added into clean porcelain dish. These porcelain dishes were placed on the hot plate at 50 degrees Celsius. There is a formation of lumps due to loss of water. After formation of damped mass, they were passed through sieve number 12 as obtained granules. These granules were dried at 60-degree Celsius.[9]

Evaluation test

1) Disintegration time

About 1gm of effervescent granules was added in 50ml water at 25 degrees Celsius. The stop watch were started. The granules when enter into water they instantly dispersed, formation of bubbles and carbon dioxide is released. When liberation of gas is stopped than, that time is consider as disintegration time.[10]

2) Amount of carbon dioxide

This method is used to determine the amount of carbon dioxide liberate from effervescent granules. weight of empty 50ml volumetric flask was taken (W1). About 50ml of prepared sulfuric acid solution was added in flask. One gram of effervescent granules was added into solution. After addition of granules there was formation of bubbles and CO2 was liberated from solution. A weight of flask after liberation of gas was taken (W2). A difference between W1 and W2 was calculate. This weight or value consider as total amount of liberation of carbon dioxide (T).[11]

By formula,

W1 + 500mg - W2 = T

Method of preparation of 50 ml sulfuric acid –

A clean and dry 50 ml volumetric flask was used. In flask 15-20 drops of water were added and then 10 ml of concentrated sulfuric acid was added. Final volume was made up to 50 ml with water.

3) pH of solution

About 1gm of effervescent granules was added in 50ml of water at 25 degrees Celsius the effervescent granules were kept in the beaker which allowed it completely to dissolve. The pH was measured using digital pH apparatus.[12]

RESULT

Evaluation test were performed on each formulation, the pH and disintegration time was shown in table

Table 2: pH and Disintegration time of effervescent granules.

Formulation Batches	pH	Disintegration time (sec)
F1	4.8	9.5
F2	4.4	9.1
F3	2.75	8.8
F4	6.1	8.5
F5	4.1	6.3
F6	4.0	6.7
F7	3.9	6.9
F8	3.5	6.8

Amount of carbon dioxide test was performed on the formulation the total liberation of carbo dioxide was shown in table 3

Table 3: Total amount of carbon dioxide gas evolved from effervescent granules.

Formulation	Weight of empty flask (W1)	Weight of flask after addition of 500mg effervescent granules	Weight of flask after liberation of gas (W2)	Total CO2 evolved
F1	85.672	86.112	86.0569	0.056 gm
F2	86.210	86.710	86.596	0.141 gm
F3	86.150	86.650	86.122	0.528 gm
F4	93.662	94.162	93.621	0.541 gm
F5	89.991	90.491	90.368	0.123 gm
F6	98.771	99.271	98.677	0.594 gm
F7	91.263	91.763	91.261	0.492 gm
F8	93.220	93.720	93.505	0.215 gm

DISCUSSION

The pH values of effervescent granules were in range between 2 to 6. This pH values of granules were considered as ideal values according to IP. The amount of carbon dioxide ranged between 0.528gm to 0.141gm for all formulations F1to F8 table 3. The formulation batches contain high amount of sodium bicarbonate and tartaric acid than produced more carbon dioxide. But the magnitude of effect for sodium bicarbonate was more than tartaric acid. At low concentration citric acid produce more carbon dioxide.

CONCLUSION

According to study it is confirm that F5 give significant result. they were prepared by using 1gm ascorbic acid, 0.1 citric acid ,0.1gm tartaric acid 1gm sodium bicarbonate ,0.203 calcium carbonate, 0.015gm sodium saccharin, 0.03gm aspartame. F5 gives pH value 4.1, disintegration time 6.3sec and amount of carbon dioxide is 0.123gm all outcomes of evaluation test within a standard range. Therefore, F5 formulation consider as an ideal formation of ascorbic acid effervescent granules.

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STABILITY STUDY OF DIFFERENT MARKETED BRANDS OF DICLOFENAC SODIUM AND PARACETAMOL TABLETS BY USING SPECTROPHOTOMETRIC METHOD

Anjan Shankar Ladage*, Umesh Rangat, Bharatee Chaudhari and Vivekkumar Redasani

YSPM'S, Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara.

***Corresponding Author: Anjan Shankar Ladage**
YSPM'S, Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara.

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ABSTRACT

The forced degradation study of different marketed formulation of combined tablets of Paracetamol and Diclofenac was performed. Paracetamol and Diclofenac was exposed to different conditions according to International Conference on Harmonization guideline. The amount of degradation product can be calculated with the help of UV spectrophotometer. The official test limits according to Indian Pharmacopoeia/United States Pharmacopoeia were considered. The marketed brands of Diclofenac Sodium and Paracetamol are Deemol-500,DIK-MR,Oxan Plus used in study as this method is less time consuming and simple and cost effective. The brands Deemol-500, DIK-MR, Oxan Plus when come in contact with different degradation parameters acid, base, and UV treatments they show negligible effects.

KEYWORDS: UV Spectroscopy, Paracetamol and Diclofenac Sodium, UV Cabinet, Degradation.

INTRODUCTION

Diclofenac Sodium and Paracetamol belongs to a class of medications known as a non-steroidal anti-inflammatory drug (NSAID) or pain killer. Combined Diclofenac Sodium and Paracetamol are widely useful for the treatment of painful musculoskeletal joint conditions like osteoarthritis, rheumatoid arthritis and ankylosing spondylitis.[1]

Diclofenac and Paracetamol contain Diclofenac (analgesic) and Paracetamol (fever reducer/mild analgesic) effective against painful musculoskeletal pain, joint pain, and skeletal muscle spasms.[2] Diclofenac works by blocking the action of a chemical messenger known as cyclooxygenase (COX) which causes pain and swelling at the injured or damaged tissue site. On the other hand, Paracetamol acts as a mild analgesic (mild pain reducer) and antipyretic (fever reducer), which enhances the pain relief action Diclofenac.[2] Additionally, it also helps to relieve toothache, ear pain, backache and other musculoskeletal related pain.

Spectrophotometric technique is based on measuring the absorption of a monochromatic light in the near ultraviolet region (200-380 nm) by colourless complex. UV spectrophotometer can also be use for stress degradation.[3] According to International Conference of Harmonization (ICH) guideline the active pharmaceutical ingredient is focused to various forced degradation conditions involves photo acid/base stress testing, temperature, photo degradation and or with humidity, time, pH variation (low and high)[4] Thermal and/or humidity stress testing is performed by exposing the drug substance to thermal/humidity conditions in due course which causes the substance to degrade forcefully to its main components.[5] UV degradation is a main trouble in frequent UV unstable products which are made up of natural and synthetic polymers as they break or disintegrate when exposed to constant sunlight. As the attack is depend on the extent and degree of exposure, nonstop exposure is a more serious problem than intermittent exposure. Acid or base stress testing is used for the evaluation of forced degradation of a drug substance.[6] This test involves degradation of a drug substance by exposure to basic or acidic medium over time to its primary degradation products. Acid or base hydrolysis occur in labile carbonyl functional groups which are esters (lactones), amides (lactams), aryl amines, carbamates, imides, imines and alcohols. Forced degradation is capable of demonstrating that the chosen technique is stability indicating that is the technique use to identify the increase in the degradation product and the subsequent loss of active components.

Simultaneous Equation Method

Simultaneous equation (SE) is typically applied to estimate drug combinations that contain two drugs or more than two drugs in combined dosage form. If the sample contains two absorbing components x & y and each absorbs at the λ_{max} of the other (λ_1& λ_2). It may be

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possible to determine both drugs by the technique of simultaneous.[7]

MATERIALS

Paracetamol and Diclofenac brands used were Deemol-500 500 mg tablets of Saint Michael Biotech, DIK-MR 500 mg tablets of Remex Healthcare Limited and Oxan Plus 500 mg tablets of Creative Remedies (AHD) Limited,1 mol/L HCl, 0.1N NaOH, reference standard Diclofenac Sodium, Paracetamol and distilled water were used, Pyrex type stirrer, measuring cylinder, pipette, beaker and volumetric flask were used, The glassware washed with distilled water, UV Cabinet, Weighing Balance, and Spectrophotometer were used in this study.

Methods

1. Preparation of standard solution of Paracetamol

30 mg pure sample of Paracetamol weighed and dissolved in 15 ml 0.1N NaOH and distilled water and final volume is adjusted with 100 ml l to obtain stock solution of 300 ug/ml, from this stock solution take 1 ml volume and diluted to 10 ml to get 30 µg/ml solution.[8]

2. Preparation of standard solution of Diclofenac Sodium

30 mg pure sample of Diclofenac Sodium weighed and dissolved in 15 ml 0.1N NaOH and distilled water and final volume is adjusted with 100 ml to obtain stock solution of 300 µg/ml, from this stock solution 1 ml volume was taken and diluted to 10 ml to get 30 µg/ml solution.[9]

3. Preparation of 0.1 N NaOH

2 gm of NaOH pellets were weighed and its solution is prepared in 500 ml volumetric flask by distilled water.[10]

4. Preparation of test/sample solution of combined tablet of Paracetamol and Diclofenac Sodium

10 tablets of combined tablet of Paracetamol and Diclofenac Sodium of Deemol-500, DIK-MR, and Oxan Plus brand were taken, weighed and average weight was calculated then triturated to form homogeneous mixture. Quantity of triturated powder equivalent to 30 mg of Paracetamol and 30 mg of Diclofenac Sodium transferred to separate 100 ml of volumetric flasks and 15ml 0.1N NaOH and distilled water was added and final volume adjusted to 100 ml then diluted to get 30 µg/ml of Paracetamol and Diclofenac Sodium respectively considered as test sample solution.[11]

5. Absorbance of standard solution of Paracetamol was taken λ_1 and λ_2.
6. Absorbance of standard solution of Diclofenac Sodium was taken at λ_1 and λ_2.
7. $^{1\%}$1cm was calculated for each at λ_1 and λ_2 and noted mean $A^{1\%}$1cm taken for calculation.
8. λ_1 and λ_2 were obtained after scanning standard solution range 400-200 nm at UV spectrophotometer.
9. Absorbance was recorded for test sample solution of combined tablet at λ_1 and λ_2.
10. Calculations were done for concentration (µg/ml) using simultaneous equation method and reported.
11. Percent purity and standard deviation were calculated and reported.
12. Final result was reported as percent purity +5.0.

Procedure for forced degradation studies

1. For acid

Forced degradation of drug substance in acidic media was performed by taking 5 ml of 30 mg/100 ml of Deemol-500, DIK-MR, and Oxan Plus in 6 separate test tubes, and then 5 ml of 1 mol/L HCl was added in each test tube. The sample was left for 30 min. Solution was transferred to a separated cuvette after the time period completion and UV absorbance of the solution was measured at the 257 nm and 275 nm wavelength.

2. For base

Forced degradation of drug substance in basic media was performed taking 5 ml of 30 mg/100 ml of Deemol-500, DIK-MR, Oxan Plus in 6 separate test tubes, then 5 ml of NaoH was added in each test tube and the sample was left for 30 min, and then UV absorbance of solution was measured at the 257 nm and 275 nm wavelength.

3. For UV light

Forced degradation of drug substance in UV light was performed by taking 5 ml of 30 mg/100 ml of Deemol-500, DIK-MR, Oxan Plus in 6 separate test tubes then 5 ml of water was added in each test tube and these test tubes were exposed to UV light for 30 min, and then UV absorbance of solution was measured at the 257 nm and 275 nm wavelength.[10]

RESULT AND DISCUSSION

We have conducted the degradation study on three brands of Diclofenac Sodium using were Deemol-500 500 mg tablets of Saint Michael Biotech, DIK-MR 500 mg tablets of Remex Healthcare Limited and Oxan Plus 500 mg tablets of Creative Remedies (AHD) Limited. When Diclofenac Sodium brands were treated with the 1 mol/L HCL, it showed availability of different brands. When Diclofenac Sodium brands were treated with the 0.1N NaOH drugs, it showed the increased availability and absorbance respectively. When exposed to UV light, changes had been observed respectively. Table 1 represents the UV absorption of different brands of the Diclofenac Sodium before and after exposing to the degradation environment. We concluded according to our results that when the Deemol-500 introduced into acidic medium 1 mol/L HCL, it showed degradation of Paracetamol that is (18.93%) and Diclofenac Sodium that is (72.73) DIK-MR showed degradation of Paracetamol (63.13%) and Diclofenac Sodium (68.50%) in acidic medium. Oxan Plus also gave greater results of Paracetamol that is (76.20%) and Diclofenac Sodium (63.62%) on exposure to acidic medium respectively. Similarly on exposure to 0.1N NaOH basic medium, the Deemol-500 showed the degradation of Paracetamol (17.43%) and Diclofenac Sodium (74.29%) respectively.

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whereas DIK-MR showed degradation of Paracetamol (60.20%) and Diclofenac Sodium (61%) while Oxan Plus gave moderate results which are Paracetamol (73%) and Diclofenac Sodium (61.33%) on exposure to basic medium respectively. When Deemol-500 exposed to UV light it gives results Paracetamol (42.48%) and Diclofenac Sodium (71.20%) respectively, whereas DIK-MR shows (64.66%) and (63%) degradation of Paracetamol and Diclofenac Sodium respectively, Oxan Plus shows (70.73%) of Paracetamol and (64%) of Diclofenac Sodium degradation when exposed to UV light for 30 min, it also showed minor changes in concentration respectively for degradation studies. Results of degradation studies are given in Tables 1 and 2.

Table 1: Absorbance of different brands of Paracetamol and Diclofenac Sodium.

Tablet	Treatment	Absorbance	
		Paracetamol	**Diclofenac Sodium**
Deemol-500	Before	λ_1=257=0.8298 λ_2=275=0.7972	λ_1=257=0.6317 λ_2=275=0.6541
	Acid Treatment	λ_1=257=0.5025 λ_2=275=0.4932	λ_1=257=0.4220 λ_2=275=0.3556
	Base Treatment	λ_1=257=0.3818 λ_2=275=0.3219	λ_1=257=0.2825 λ_2=275=0.2522
	UV Treatment	λ_1=257=0.5620 λ_2=275=0.4820	λ_1=257=0.5180 λ_2=275=0.4280
DIK-MR	Before	λ_1=257=0.6888 λ_2=275=0.6532	λ_1=257=0.7312 λ_2=275=0.6920
	Acid Treatment	λ_1=257=0.6620 λ_2=275=0.6432	λ_1=257=0.4212 λ_2=275=0.3813
	Base Treatment	λ_1=257=0.4020 λ_2=275=0.3813	λ_1=257=0.3930 λ_2=275=0.3720
	UV Treatment	λ_1=257=0.6020 λ_2=275=0.5813	λ_1=257=0.5520 λ_2=275=0.5218
Oxan Plus	Before	λ_1=257=0.7810 λ_2=275=0.7220	λ_1=257=0.7312 λ_2=275=0.6810
	Acid Treatment	λ_1=257=0.7030 λ_2=275=0.3820	λ_1=257=0.6030 λ_2=275=0.6820
	Base Treatment	λ_1=257=0.4513 λ_2=275=0.4130	λ_1=257=0.5820 λ_2=275=0.5412
	UV Treatment	λ_1=257=0.6614 λ_2=275=0.6413	λ_1=257=0.6830 λ_2=275=0.6520

Table 2: Absorbance of different brand of Paracetamol and Diclofenac Sodium in percentage.

Tablet	Treatment	Absorbance	
		Paracetamol	**Diclofenac Sodium**
Deemol-500	Before	57%	75.11%
	Acid Treatment	18.93%	72.73%
	Base Treatment	17.43%	74.29%
	UV Treatment	42.48%	71.20%
DIK-MR	Before	75.11%	70.03%
	Acid Treatment	72.73%	68.50%
	Base Treatment	74.29%	61.05%
	UV Treatment	71.20%	63.20%
Oxan Plus	Before	78%	66.15%
	Acid Treatment	76.20%	63.62%
	Base Treatment	73%	61.33%
	UV Treatment	70.23%	64.10%

In present study the stability testing was performed as per ICH guidelines. The degradation like Acid degradation, Base degradation and UV degradation were performed and trace degradation was found.

CONCLUSION

It was used to study the degradation as per ICH guideline. Paracetamol and Diclofenac Sodium was found to be degraded in almost all type of condition (acidic, basic and uv light) Degradation of different brands of Paracetamol and Diclofenac Sodium were

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carried out in different condition showed more changes in different medium.

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POST MARKET IN-VITRO QUALITY CONTROL EVALUATION FOR DIFFERENT BRANDS OF PARACETAMOL TABLETS AVAILABLE IN INDIAN MARKET

Kaustubh Jagtap*, Bharatee Chaudhari, Aishwarya Jadhav and Vivekkumar Redasani

YSPM'S, Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara.

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***Corresponding Author**
Kaustubh Jagtap
YSPM'S, Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara.

ABSTRACT

Paracetamol scientifically known as acetaminophen is quite popular over-the-counter (OTC) form of analgesic and antipyretic. It is widely used in most of the countries. Acetaminophen is active metabolite of phenacetin. Chemically, it is 4-hydroxy acetanilide (acetaminophen). Paracetamol has medical uses such as it is commonly used for the relief from headache, minor pains, aches and is a major ingredient in numerous cold and flu remedies. It can be used in all ages of people for reducing fever. The main objective of this case study is to check and compare the quality of marketed finished product of paracetamol tablet formulation which are locally and commonly available in Indian pharmaceutical market manufactured by various pharmaceutical companies as India is one of the biggest pharmaceutical product producers in the world. This study includes the randomly selected four different brands (A, B, C, D) as Calpol 500, Pyrigesic, Pacimol 500 and Febrex of paracetamol conventional tablets of 500 mg strength of active pharmaceutical ingredient of paracetamol from local medical pharmacy stores. These different brand tablets were compared by the in-vitro test accordingly the test procedure given in IP and USP standards and unofficial test standards which are also integral part of this quality control tests. The test parameters for quality assessment and evaluation of tablets are weight variation i.e., weight uniformity, friability, hardness, disintegration time and drug assay content by UV spectrophotometer were performed as per standard of pharmacopoeias. The results are then formed according to the limit ranges of pharmacopeial standards.

KEYWORDS: Paracetamol, Quality Control, Weight Variation, Disintegration Time, Drug Assay.

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1. INTRODUCTION

Paracetamol or acetaminophen is active metabolites of phenacetin. It is a widely used over-the-counter analgesic and antipyretic. Chemically, it is 4-hydroxy acetanilide (acetaminophen). Paracetamol is approved for reducing fever in people of all ages. It is commonly used for the relief of headaches, other minor aches and pains, and is a major ingredient in numerous cold and flu remedies. It is classified as a non-steroidal anti-inflammatory drug (NSAID) by some sources and not as an NSAID by others, while most sources implicitly distinguish them, for example by mentioning both NSAIDs and paracetamol in the same sentence. Paracetamol has few anti-inflammatory effects in comparison to NSAIDs. Paracetamol is available in different dosage form: tablet, capsules, drops, elixirs, suspension and suppositories.[1,2]

The concept of total energy quality control refers to the produce a perfect product by a series of measures requiring an organized effort by the entire company to prevent or eliminate errors at every stage in production. Although the responsibility for assuring product quality belongs principally to quality assurance personnel, it involves many departments and disciplined lines within a company. To be effective, it must be supported by a team effort. Quality must be built into a drug product during product and process design, and it is influenced by the physical plant design, space, ventilation, cleanliness, and sanitation during routine production. The product and process design begins in research and development, and includes preformulation and physical, chemical, therapeutic, and toxicologic considerations.[3,4]

The assurance of product quality depends on more than just proper sampling and adequate testing of various components and the finished dosage form. Prime responsibility of maintaining product quality during production rests with the manufacturing department.[3,4]

For the conventional tablets weight variation, friability, disintegration, dissolution, drug assay, uniformity of contents is the evaluation test those are required to perform to confirm about the quality of tablet. Friability is the tested for a tablet to see whether the tablet is stable to abrasion or not, it is tested by using Roche friabilator and 1% maximum loss in the weight after friability test is allowed. Weight variation test is performed to check that the manufactured tablets have a uniform weight. Disintegration test is performed to see how much time a tablet takes to break down in to the small particles. The drug assay study

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provides the information how much practically available in the given dosage form and after comparing with the theoretical value, a result about the efficacy can be given.[3,4,5]

2. MATERIALS

For the present case study different paracetamol tablets brands were used such as Calpol 500 mg tablets of GlaxoSmithKline Pharmaceutical Limited, Pyrigesic 500 mg tablets of East India Pharmaceutical Works Limited, Pacimol 500 mg tablets of Ipca Laboratories Limited and Febrex Indoco Remedies Limited. 0.1M NaOH, reference standard Paracetamol, double distilled deionized filtered water is used. Pyrex type stirrer, measuring cylinder, pipette, funnel, beaker and volumetric flask, petri dish, cuvettes, butter paper, Whatman filter paper No. 44, spatula, tissue paper were used. Freshly laboratory prepared distilled water was used to wash glasswares. Wensar high precision balance, Friabilator, Disintegrator, Monsento hardness tester, Shimadzu UV spectrophotometer, Systronics UV vis double beam spectrophotometer.

3. METHODS

3.1. Weight variation test

Weigh individually randomly selected 20 tablets and calculate the weight of each individual tablet. Not more than 2 tablets of individual weight deviates than the average weight by the percentage given as per Indian Pharmacopoeia limits. The process repeated same for each brand of conventional paracetamol tablets.[6,7]

3.2. Friability test

For each of the brands, 10 tablets were selected and carefully dusted before testing and weighed. Then the tablets were placed in the drum of friability tester and rotated at the speed of 25rpm for 4 minutes. After 100 revolutions and de-dusting, tablets were re-weighed and the friability percentage was calculated by the following equation:[6,7,8]

Formula: $$\% \text{ Friability} = \frac{\text{Initial weight } (W_1) - \text{Final weight } (W_2)}{\text{Initial Weight } (W_1)} \times 100$$

3.3. Disintegration test

Six tablets were randomly selected from each brand and placed in the disintegration apparatus, which is filled by 900 mL of distilled water (disintegration medium) maintained at 37±1°C. The time taken to disintegrate the tablet and pass through the mesh was recorded and

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the mean of time taken was calculated. The process repeated same for each brand of conventional paracetamol tablet.[6,7,8]

3.4. Hardness test

To conduct the hardness test, 10 tablets of each brand were randomly selected and the crushing strength of the tablets was measured by using instrument Monsento hardness tester. The average hardness of the tablet was calculated. The process repeated same for each brand of conventional paracetamol tablet.[8,9]

3.5. Assay of paracetamol

Weigh and powder 20 tablets. A quantity of powder containing about 0.15 mg of paracetamol, add 50 ml of 0.1M NaOH, dilute with 100 ml of water, shake for 15 minutes add sufficient water to produce 200 ml. Mix, filter and dilute 10.0 ml of filtrate to 100 ml with water. To 10.0 ml of resulting solution add 10 ml of 0.1M NaOH, dilute to 100.0 ml with water and mix. Measure the absorbance of the resulting solution at maximum at about 257 nm. Calculate the content of $C_8H_9NO_2$ taking 715 as specific absorbance at 257 nm.[10]

4. RESULT AND DISCUSSION

After random selection of tablets, the weight variation, friability, disintegration time, hardness and drug assay were performed as per the Indian Pharmacopoeia and United States Pharmacopoeia.

4.1. Weight variation test

The result of weight variation of twenty randomly selected tablets of each brand are as follows:

Table 1: Weight variation test for four different brands of paracetamol tablets.

No. of tablets	Calpol 500 (A) Avg. Weight = 629 mg			Pyrigesic (B) Avg. Weight = 568 mg		
	Weight (mg)	Weight Variation	% Weight Variation	Weight (mg)	Weight Variation	% Weight Variation
1	662 mg	7	1	569 mg	1	0.17
2	628 mg	1	0.15	567 mg	1	0.17
3	626 mg	3	0.47	566 mg	2	0.35
4	629 mg	0	0	580 mg	12	2.11
5	629 mg	0	0	563 mg	5	0.88
6	633 mg	4	0.63	566 mg	2	0.35
7	629 mg	0	0	565 mg	3	0.52
8	624 mg	5	0.79	573 mg	5	0.88

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9	630 mg	1	0.15	566 mg	2	0.35
10	630 mg	1	0.15	570 mg	2	0.35
11	631 mg	2	0.31	576 mg	8	1.40
12	622 mg	7	1	571 mg	3	0.52
13	625 mg	4	0.63	563 mg	5	0.88
14	627 mg	2	0.31	556 mg	12	2.11
15	635 mg	6	0.95	576 mg	8	1.40
16	638 mg	9	1.43	569 mg	1	0.17
17	634 mg	8	1.27	576 mg	8	1.40
18	645 mg	18	2.54	571 mg	3	0.52
19	626 mg	3	0.47	564 mg	4	0.70
20	631 mg	2	0.31	570 mg	2	0.35

No. of Tablets	Pacimol 500 (C) Avg. Weight = 560 mg			Febrex (D) Avg. Weight = 581 mg		
	Weight (mg)	Weight Variation	% Weight Variation	Weight (mg)	Weight Variation	% Weight Variation
1	569 mg	9	1.60	581 mg	0	0
2	565 mg	5	0.89	581 mg	0	0
3	567 mg	7	1.25	577 mg	4	0.68
4	557 mg	3	0.53	579 mg	2	0.34
5	569 mg	9	1.60	579 mg	2	0.34
6	542 mg	18	3.21	583 mg	2	0.34
7	563 mg	3	0.53	593 mg	12	2.06
8	558 mg	2	0.35	581 mg	0	0
9	562 mg	2	0.35	586 mg	5	0.86
10	569 mg	9	1.60	579 mg	2	0.34
11	557 mg	3	0.53	586 mg	5	0.86
12	564 mg	4	0.71	580 mg	1	0.17
13	559 mg	1	0.71	575 mg	6	1.03
14	558 mg	2	0.35	583 mg	2	0.34
15	560 mg	0	0	576 mg	5	0.86
16	559 mg	1	0.17	577 mg	4	0.68
17	555 mg	5	0.89	589 mg	8	1.37
18	553 mg	7	1.25	585 mg	4	0.68
19	560 mg	0	0	583 mg	2	0.34
20	557 mg	3	0.53	567 mg	14	2.40

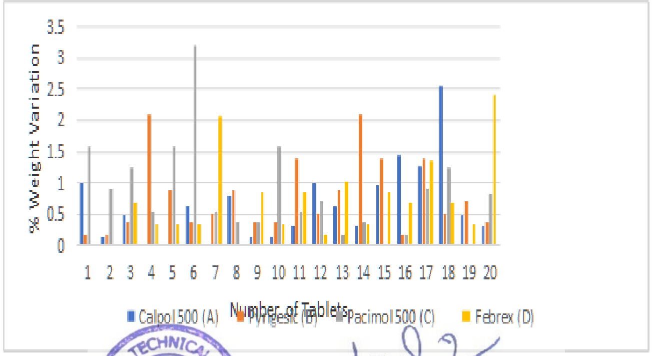

Figure 1: Weight variation test for four different brands of paracetamol tablets.

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As per the IP the weight variation limit for the tablet which is having the weight equal or more than 250 mg is ±5 % and the given results shown that all the twenty randomly selected tablets of all four brands are having weight variation less than ±5 % which proves that the four brands (A, B, C, D) of paracetamol tablets those are available in the Indian pharmaceutical market passed the official weight variation test.

4.2. Friability test: The friability test was conducted in Roche friabilator by using 10 tablets, the results of all different brands are as follows:

Table 2: Friability test for four different brands of paracetamol tablets.

Brand	Calpol 500 (A)	Pyrigesic (B)	Pacimol 500 (C)	Febrex (D)
% Friability	0.61%	0.77%	0.87%	0.27%

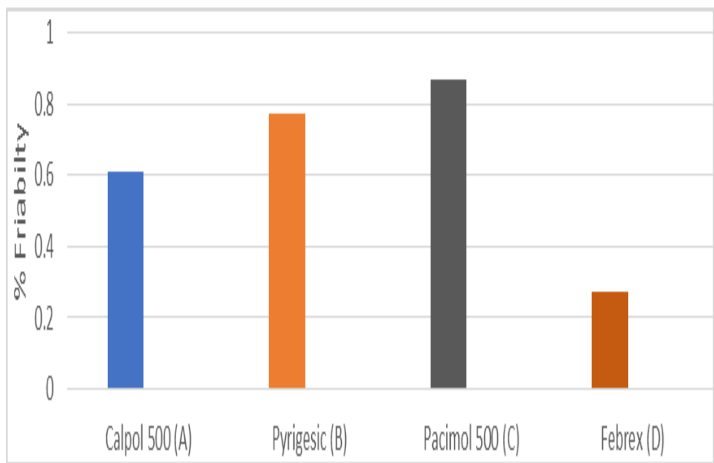

Figure 2: Friability test for four different brand of paracetamol tablets.

The results of friability test shows that all the four brands of paracetamol tablets for Calpol 500 (A) is 0.61%, Pyrigesic (B) is 0.77%, Pacimol 500 (C) is 0.87% and Febrex (D) is 0.27% which are under the pharmacopoeia limits 1% means as per IP standard. All these brands of paracetamol tablets those are available in Indian pharmaceutical market are having good strength and can tolerate the shocks during transportation handling of these tablets.

4.3. Disintegration test: The disintegration test was performed in the distilled water at 37 ±2°C in the Almicro Disintegration instrument. The results of all four brands are as follows:

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Table 3: Disintegration time for four different brands of paracetamol tablets.

Brand	Calpol 500 (A)	Pyrigesic (B)	Pacimol 500 (C)	Febrex (D)
Disintegration time	1 min.	2:49 min.	2 min.	9 min.

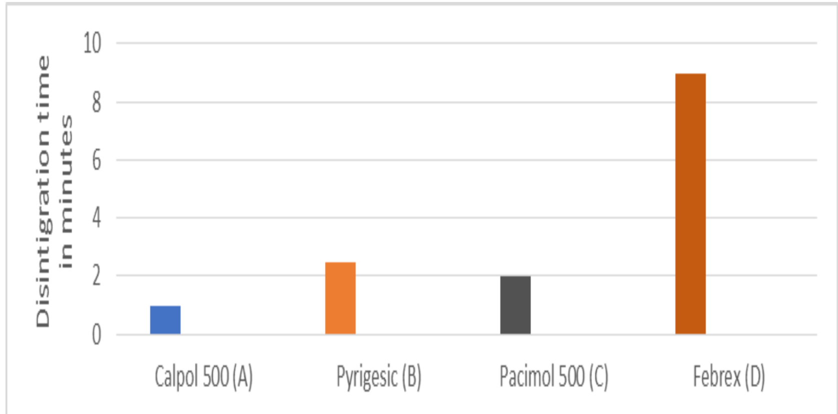

Figure 3: Disintegration time for four different brands of paracetamol tablets.

The results of disintegration test shows that all four different brands of paracetamol are as Calpol 500 (A) is 1 min., Pyrigesic (B) is 2:49 min., Pacimol 500 (C) is 2 min. and Febrex (D) is 9 min. tablet disintegration time is less than the standard disintegration time (15 minute) for uncoated tablet as per IP standards which proves that all these brands of paracetamol tablet pass the quality control limits as per the pharmacopoeia. The brand A disintegration time is about 1 min. means it disintegrates very fast so it might be possible that the drug will be available very fast for absorption as well as the onset of time will be very less.

4.4. Hardness test: The hardness test is conducted with Monsanto hardness tester by using 10 tablets, the results of all different brands as follows:

Table 4: Hardness test for four different brands of paracetamol tablets.

Brand	Calpol 500 (A)	Pyrigesic (B)	Pacimol 500 (C)	Febrex (D)
Hardness Kg/cm^2	6.2 kg/cm^2	7.6 kg/cm^2	10.2 kg/cm^2	11.4 kg/cm^2

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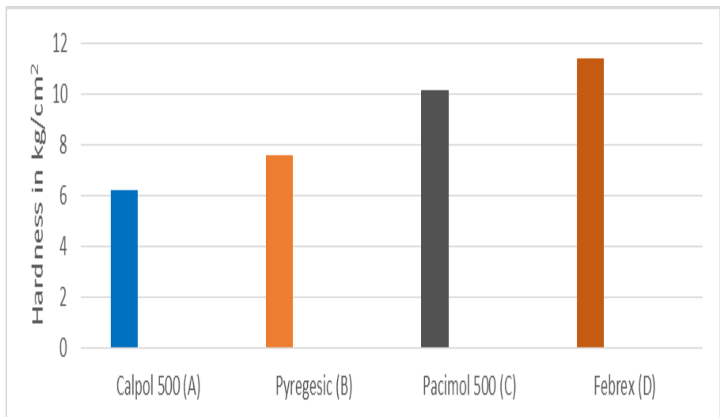

Figure 4: Hardness test for four different brand of paracetamol tablets.

The result of hardness test for all four brands of paracetamol tablets are Calpol 500 (A) is 6.2 kg/cm^2, Pyrigesic (B) is 7.6 kg/cm^2, Pacimol 500 (C) is 10.2 kg/cm^2, Febrex (D) is 11.4 kg/cm^2. The limit for hardness as per USP is 4 to 10 kg/cm^2 as per the pharmacopoeia the range of hardness closely near to 10 kg/cm^2 can be approved to pass the test. High crushing strength is attributed to a high compression force, high binder concentration or excess volume of granulating fluid. Although all uncoated brands of Paracetamol tablets have very high hardness, they still exhibited very good quality control parameters such as dissolution profile, disintegration time and chemical content determination. This indicates that hardness test is not a critical quality control parameter.

4.5. Drug assay content: To confirm the amount of paracetamol drug in the tablet drug assay was performed for all four different brands, the results are as follows:

Table 5: Drug assay content for four different brands of paracetamol tablets.

Brand	**Calpol 500 (A)**	**Pyrigesic (B)**	**Pacimol 500 (C)**	**Febrex (D)**
%Drug content	95.13%	97.20%	102%	98%

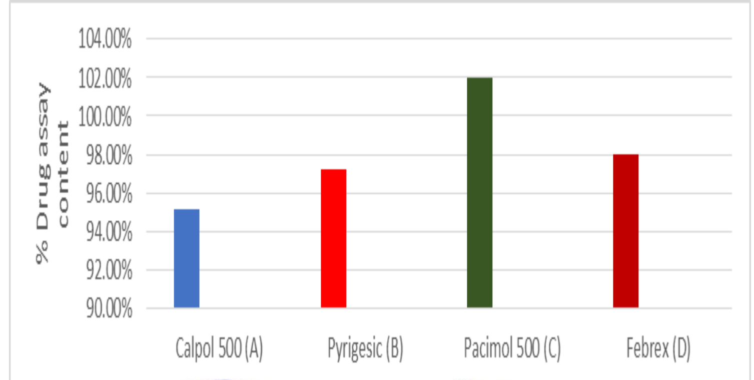

Figure 5: Drug assay content of four different brands of paracetamol tablets.

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The results of drug assay of four different brands of paracetamol tablets are Calpol 500 (A) is 95.13%, Pyrigesic (B) is 97.20%, Pacimol 500 (C) is 102% and Febrex (D) is 98%. It shows that amount of paracetamol drug available in all these formulations is between 95% to 105% as per IP standard range it means drug are available as per pharmacological action. There was no statistically significant difference between the different brands of the paracetamol tablets. Furthermore, all the brands of the tablets passed the test for the content of paracetamol.

CONCLUSION

As post market evaluation of approved medicines is essential to monitor, it will meet the desired standards of quality, safety and therapeutic efficacy of medicine for the consumers. For present case study all quality control test for four different brands of conventional paracetamol tablets available in Indian market were assessed. In present case study different quality control parameters were studied such as weight variation, friability, disintegration time, hardness and drug assay content. All values will be compared with the standards of Indian Pharmacopoeia and United States Pharmacopoeia. This study revealed that all brands of paracetamol tablets met the IP and USP specifications.

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TRADITIONAL HERBAL SYRUP: A REVIEW

Snehal Mahamuni*, Bharatee Chaudhari and Vivekkumar Redasani

YSPM's Yashoda Technical Campus, Faculty of Pharmacy, NH-4, Wadhe, Satara – 415011.

***Corresponding Author: Snehal Mahamuni**
YSPM's Yashoda Technical Campus, Faculty of Pharmacy, NH-4, Wadhe, Satara - 415011.

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ABSTRACT
Syrups, solutions, emulsions, or suspensions containing one or more active ingredients in an appropriate vehicle are examples of liquid oral formulations. Some oral liquid formulations are made by diluting concentrated liquid preparations for drops or syrups in a suitable vehicle. Syrups are aqueous formulations with a sweet flavor and the appropriate viscosity. A suitable combination of polyols, sweetening agents, aromatic, and flavoring agents can be used to achieve an acceptable sweet taste. The stability of the active and inactive ingredients is a significant concern for the formulator in liquid oral formulation. Active ingredients are typically lower stability in aqueous formulations than in solid dosage forms. As a result, it is critical to stabilize and preserve the water-containing liquid oral formulation. Herbal medicine refers to the use of extract for therapeutic purposes, and the majority of herbal syrup was initially obtained from plants. In addition to alternative dosages from natural medications, herbal syrups were also developed. Herbal syrup is now utilized to treat a variety of conditions and to alleviate disease symptoms. Herbal syrup is characterized as a prepared, combined, and concentrated decoction with honey sugar or, on occasion, alcohol. The base of this syrup is a strong herbal decoction, which is thickened and preserved by mixing it with sugar honey. Herbal plants and formulations are used to treat a number of illnesses, like as cough syrup and other illnesses. This review discusses the extraction processes, standardization, phytochemical analysis and evaluation parameters of herbal syrup.

INTRODUCTION
Herbal Syrup: Herbal syrup it is a defined as a prepared and combination and concentration decoction with Honey sugar or either some time use alcohol. The base of such a syrup is a powerful herbal decoction, and thickening it with sugar honey helps to preserve it. Herbal plants and formulations are used to treat a variety of ailments, including cough syrup and other illnesses. Many varieties of herbal plants are utilized for cough syrup, including pudina, Tulsi, Cinnamon, and honey, and the entire plant has been used for manufacturing herbal medicine for many years.

Herbal formulation is the most often used method of health treatment in both developed and developing countries. The cough syrup medication is a liquid dose form, and the use of oral liquid pharmaceuticals has been confirmed on the basis of basic simplicity of administration to those who have difficulty swallowing solid prescription dosages. Syrup is a concentrated solution made of sugar and pure water. In syrup, as opposed to other types of syrup solutions.

The syrup may or may not contain medication or a flavoring agent mixture. Flavored or non-medicated syrup is syrup that does not contain medication but does contain a flavoring agent. Flavored syrup is frequently used as a vehicle for the unpleasant test results of medications (found as) in medicated syrups. The presence of syrup in high concentrations predisposes them to bacterial infection, so they frequently as a preservative.[1]

Syrups are a popular delivery vehicle for anti-tumor medications because they are easier to swallow (ingest) than tablets and capsules. This medication is quickly noticed. There are synthetic cough preparations available, but they have a number of negative side effects. As a result, the current study demonstrated that violet herbal cough syrup contains natural elements with no side effects. In general, health professionals are having difficulty accessing effective and safe natural treatments (therapy). A number of allopathic medication products have not been studied on a large scale, and they are generally solid without knowledge of their mechanism of action or side effects.

Even though the use of complementary medication is sometimes helpful and the confirmation of the effectiveness of some of this all-medication literature is limited, they are frequently sold with the drug store.[2] A successful formulation of liquid as well as other dosage forms necessitates a combination of scientific acumen and pharmaceutical "art".[3] Because harmful changes

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occur more easily in solution, oral liquid medicines are gradually being replaced by tablets and capsules. Nonetheless, a large number of liquid oral preparations are still available in the official books.

In fact, the absorption of medicaments in solution from the GI tract into the systemic circulation is expected to be faster than that of other oral dosage forms of the same medicinal agent. Ayurvedic formulations are typically administered orally, and the majority of orally administered Ayurvedic formulations are in the form of a liquid drug or drug combination. Herbal medicinal combination, on the other hand.

Types of herbal syrup

1. Flavored syrup
2. Medicated syrup
3. Artificial syrup.[4]

Herbal syrup is manufactured by combining a concentrated decoction of herbs with honey or sugar, as well as alcohol. The herbal syrup is created through a decoction process. By combining a herb decoction with sugar, the formulation can be thickened and preserved. This was responsible for extending the shelf life of the formulation. The addition of sweetener can also help to improve the palatability of some herbs. The syrup that was eventually obtained was delicious. As defined, it is a thick, sticky liquid made up of a concentrated solution of sugar and water, with or without the addition of flavorings, agents, or medicinal substances.[5]

Although most traditional healthcare systems are effective, they lack proper standardization. Standardization is an crucial step in establishing consistent biological activity, a specified chemical profile, or just a quality assurance programme for herbal formulation production and manufacturing. As a prerequisite for global harmonization, WHO has issued specific guidelines for assessing the safety, efficacy, and quality of herbal medicines. As a result, a polyherbal syrup was created by combining dried powder decoctions of various herbs. The current study includes the standardization of raw materials for their identity, quality, and development of polyherbal syrup, as well as the standardisation of the produced formulation and accelerated stability studies.[6]

IDENTIFICATION, EVALUATION AND STANDARDIZATION OF CRUDE DRUGS

In recent era, there has been great demand for plant derived products in developed countries. These products are increasingly being sought out as medicinal products, nutraceuticals, and cosmetics. There are around 6000 herbal manufacturers in India. Ayurvedic medications are manufactured in about 4000 units. World Health Organization (WHO) provide guidelines for the herbal standardization and analysis of herbs. WHO Guidelines for Herbal Drug Standardization and Evaluation The WHO guidelines for herbal drugs can be summarized as follows:

1. Identity of the drug: Botanical evaluation- sensory characters, foreign organic matter, microscopical, histological, histochemical evaluation, quantitative measurements etc.
2. Physicochemical character of the drug: Physical and chemical identity, Chromatographic fingerprints, ash values, extractive values, moisture content, volatile oil and alkaloidal tests, quantitative estimation techniques, and so on.
3. Pharmacological parameters, biological activity profiles, bitterness values, hemolytic index, astringency, swelling factor, foaming index etc.
4. Toxicity details: - pesticide residues, heavy metals, microbial contamination like total viable count, pathogens like E. coli, Salmonalla, P.aeroginosa, S. aureus, Enterobacteria etc.
5. Microbial contamination.
6. Radioactive contamination.[7]

Herbal Drugs: The herbal drugs define as whole or plants parts, algae, fungi in unprocessed state usually in dried form but sometimes fresh. Because of the ever-increasing use of plant-based medicines and the rapid growth of the global market for these products, the safety and quality of medicinal plant materials and final herbal medicines has become a major issue for the public health establishment.[8] There is significant diversity in the quality management of such materials and products, which has an influence on population health as contaminants in herbal medicines may represent preventable dangers It has implications for consumers, as well as international trade.[9]

The International Conference of Drug Regulatory Authorities (ICDRA) and the National Centres participating in the WHO Drug Monitoring Programme asked WHO to develop and continuously revise technical guiding principles on quality, safety, and efficacy of herbal medicines in order to reduce the risk of adverse events caused by precarious and low-quality herbal medicines. The process of standardisation is concerned with the physicochemical analysis of crude medication, completed product safety, effectiveness, and consistency evaluation, safety and risk qualifications based on experience, consumer product information stipulation, and product endorsement.[10]

Because polyherbal formulations combine more than one herb to provide the ideal therapeutic effect, evaluation is critical for maintaining the quality and safety of the product. It decreases batch-to-batch variation and assures the efficacy, safety, quality, and sufficiency of polyherbal formulations. This is accomplished by limiting the intrinsic divergence of natural product composition through the application of quality assurance practises to agricultural manufacturing procedures should take into contemplation each and every one phase that adds to the quality of the herbal drugs, specifically

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accurate identity of the sample, organoleptic assessment, pharmacogenetic study, volatile matter, quantitative analysis, phytochemical evaluation test for the presence of xenobiotics, microbial load testing, toxicity study and biological activity.[11]

The various parameters for identification, evaluation and standardization.[7,12]

Sr.No.	Methods	Evaluation Parameters
1.	Authentication	A. Parts of plants collect like leaf, flower, root, stolen B. Regional status C. Family D. Biological source E. Chemical constituents
2.	Marphology or Organoleptic evaluation	A. Odour B. Taste C. Size D. Shape E. Special feature
3.	Microscopy evaluation	A. Leaf content B. Trichomes C. Stomata D. Quantitative microscopy
4.	Chemical evaluation	A. Chemical test B. Chemical assay C. Phytochemical screening
5.	Physical evaluation	A. Moisture content B. Viscosity C. Melting point D. Solubility E. Optical rotation F. Refractive index G. Ash value H. Extractive value I. Volatile oil content J. Foreign matter etc.
6.	Biological evaluation	A. Microbial contamination B. Pesticides contamination C. Pharmacological activity of drugs

EXTRACTION TECHNIQUES

Maceration, digestion, percolation, infusion, decoction, hot continuous extraction (Soxhlet), counter current extraction, aqueous-alcoholic extraction by fermentation, supercritical fluid extraction, microwave-assisted extraction, ultrasound extraction (sonication), and distillation techniques (steam distillation, water distillation, phytonic extraction) are all examples of medicinal plant extraction techniques (with hydro fluorocarbon solvents). Hydro water and steam distillation), hydrolytic maceration followed by distillation, expression, and effleurage (cold fat extraction) are all options for aromatic plants. Headspace trapping, solid phase micro extraction, protoplast extraction, and micro distillation are some of the most recent aromatic plant extraction technologies.[13]

1. **Plant tissue homogenization:** Researchers have employed homogenization of plant tissue in a solvent extensively. Fresh plant components are ground to fine powder in a blender, then mixed with a specific amount of solvent and shaken rapidly for 5 to 10 minutes or after 24 hours, the extract is filtered after that.. To evaluate the concentration, the filtrate can be dried under decreased pressure and redissolved in the solvent. However, other researchers centrifuged the filtrate to clarify the extract.[14]
2. **Serial exhaustive extraction:** Another popular extraction approach comprises sequential extraction with changing polarity solvents to assure that a wide polarity range of components can be extracted, from a non-polar (hexane) to a more polar (methanol). Some researchers use an organic solvent to do soxhlet extraction of dried plant material. This approach is not suitable for thermolabile chemicals because prolonged heating may cause degradation.[14]
3. **Soxhlet extraction:** When the target molecule has a low solubility in a solvent in which the impurity is insoluble, soxhlet extraction is required. If the desired component has a high solubility in a solvent, it can be separated from the insoluble substance using simple filtration. The advantage of this approach is that instead of passing multiple batches of warm solvent through the sample, only one batch

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is recycled. This approach is not suitable for thermolabile chemicals since prolonged heating can cause degradation.[15]

4. **Maceration:** In maceration (for fluid extract), whole or grinded plant-drugs are held in contact with the solvent in a tight-fitting container for a set period of time, with regular agitation, until all soluble materials is dissolved. This approach is most effective when dealing with thermolabile pharmaceuticals.[16]
5. **Decoction:** This method is used for the extraction of the water soluble and heat stable constituents. This process involves boiling a crude medication in water for 15 minutes, chilling, filtering, and pouring enough cold water through it to generate the required volume.[17]
6. **Infusion:** It is a dilute solution of the crude medications' readily soluble components. Fresh infusions are made by macerating materials in cold or hot water for a small time period.[17]
7. **Digestion:** This is a type of maceration in which the maceration extraction process is heated gently. When a relatively raised temperature is not undesirable and the menstrual solvent efficiency is increased, it is employed.[17]
8. **Percolation:** In the production of tinctures and fluid extracts, this is the method most commonly used to extract active substances. In most cases, a percolator (a thin, cone-shaped jar with openings on both ends) is utilised. The solid materials are soaked with an adequate amount of the prescribed menstruum and let to stand for around 4 hours in a tightly sealed container, following which the mass is compressed and the percolator's lid is closed. A shallow layer of menstruum is poured above the mass, and the combination is macerated for 24 hours in a closed percolator. The percolator's outlet is then opened, allows the inside liquid to gradually drop out. As required, menstruum is added more and more till it percolate reaches approximately three-quarters of the finished product's volume. After pressing the marc, the liquid is poured into the percolate. The required amount of menstruum is added, and the mixed liquid is purified by filtration or standing followed by decanting.[18]
9. **Sonication:** Frequency of ultrasound waves ranging from 20 to 2000 kHz are used in the technique, which enhances the permeability of cell walls and causes cavitation. Although the method is effective in particular situations, such as rauwolfia root extraction, its use on a broad scale is limited due to the increased costs. One downside of the process is the known but rare adverse effect of ultrasonic energy (more than 20 kHz) on the active ingredients of medicinal plants, resulting in the production of free radicals and, as a result, unwanted alterations in the drug molecules.[18]

PHYTOCHEMICAL ANALYSIS

Phytochemical examination as per the standard methods:[13,19]

Sr.No.	Phytoconstituent	Test
1.	Alkaloids	Mayer's test
		Dragendorff's test
		Wagner's test
		Hager's test
2.	Glycosides	Legal's test
		Keller-killiani's test
		Borntrager's test
3.	Carbohydrates	Molisch's test
		Fehling's test
		Benedict's test
4.	Tannins	Ferric chloride test
		Gelatin test
		Lead acetate test
5.	Phytosterols	Liebermann-burchard's test
		Salkowski's test
6.	Reducing sugars	Fehling's test
		Benedict's test
7.	Flavonoids	Ferric chloride test
		Shinoda test
		Alkaline reagent test
		Lead acetate test
8.	Saponins	Foam test
9.	Proteins and amino acids	Biuret test
		Ninhydrin test

EVALUATION PARAMETRS

1. Colour

The syrup's colour is examined directly with our naked eye.[20,21]

2. Odour

Individually, 5ml of final syrup was smelled, and the odour was identified.[20,21]

3. Taste

To determine the taste, a pinch of the final syrup was placed on the tongue's taste bud.[21]

4. Determination of pH

Take 10ml of final syrup in the volumetric flask and fill up the volume upto 100ml with distilled water. A digital pH meter was used to measure the pH.[21,22]

5. Determination of viscosity

The viscosity of syrup can be measured using an ostwald viscometer. First, carefully clean the ostwald viscometer with warm chromic acid or acetone. Fill the water up to the mark "G" in the dry viscometer and place the viscometer vertically on a suitable platform. Take note of the time it takes for water to flow from mark A to mark B. Repeat the filling operation at least three times and record the time to acquire reliable readings. Now rinse the viscometer and fill it with test liquid (syup) till mark A, then calculate the time it takes for the liquid to flow to

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mark B. A specific gravity bottle can be used to determine density.[22,23]

Formula for viscosity

Viscosity = (Density of test liquid × Time required to flow test liquid × Viscosity of water) / (Density of water × time required to flow water)

6. Determination of density

The density of syrup can be calculated using the specific gravity of the bottle. Use chromic acid or nitric acid to thoroughly clean the specific gravity bottle. Rinse the bottle two to three times with distilled water. Take note of the weight of the empty dry bottle with the capillary tube stopper (w1). Fill the bottle with unknown liquid, close it, and wipe the excess liquid out of the bottle with unknown liquid in analytical balance (w2). Finally, compute the weight in grammes of an unknown liquid (w3).[23]

Formula for density:

Density of liquid under test (syrup) = Weight of liquid under test / Volume of liquid under test

= w3/v

7. Determination of specific gravity

After cleaning with chromic acid or nitric acid, rinse the bottle with filtered water two to three times. If necessary, rinse and dry the bottle. Take the weight of an empty dry bottle with a capillary tube stopper (w1). Fill the bottle with distilled water, screw on the stopper, and wipe away any surplus liquid from the outside of the tube. And, using an analytical balance, weigh the bottle with distilled water (w2). After emptying and drying, repeat the procedure by replacing water with the liquid under test (syrup). Weigh the container with the stopper and the liquid under test on an analytical balance (w3).[23]

Formula for specific gravity

Specific gravity of liquid under test (syrup) = Weight of liquid under test / Weight of water.

= w3/w2.

ADVANTAGES OF HERBAL SYRUP

1. Production costs are low.
2. With chronic conditions, it is effective.
3. Various options are available.
4. They could have less negative side effects.
5. It's simple to adapt the dose to the weight of the child.
6. There is no need for nursing.
7. They are usually harmless.
8. Herbs can be found almost anywhere.
9. As a syurp is sweet in flavour, it's good patient complimac, especially for paediatric patients.
10. Because of the high osmotic pressure, it acts as a preservative by inhibiting the growth of bacteria, fungi, and mould.

DISADVANTAGES OF HERBAL SYRUP

1. There are no dosing instructions.
2. Wild herbs provide a risk of poisoning.
3. Solid sedimentation occasionally results in the formation of a foot.
4. It is impossible to attain dose precision unless the syrup is packaged in unit doses.
5. If preservation is not added in the correct proportion, microbiological contamination can occur.
6. An additional drawback of herbal medication is the risk of self-dosing of herbs, which is very rare.

CONCLUSION

In today's world, herbal products are a symbol of safety, as contrast to synthetic pharmaceuticals, which are considered unsafe to both humans and the environment. Herbs have been valued for decades for their medicinal, flavouring, and aromatic properties. When designing a herbal medication formulation, it is essential to have a complete knowledge of the drug's organoleptic properties, phytoconstituents, pharmacological action, and standardisation in relation to numerous parameters using various approaches.

Monographs, which are compiled in standard books such as the Indian Pharmacopoeia, Ayurvedic Pharmacopoeia of India, Wealth of India, and Ayurvedic Formulary, provide all the details for the various tests to be performed in order to determine the conformity of the crude or formulated herbal drug with the standards established. The involved governing authorities, such as CDSCO and the US-FDA, have established numerous rules on the standards of herbal pharmaceuticals, as well as standard testing techniques to determine the drug's conformity with prescribed standards.

The herbal syrup is a sweet, viscous, concentrated, or a nearly saturated aqueous solution of sucrose-containing 66.7% w/w of sugar (USP contains 64.74%w/v of sugar) having a specific gravity of 1.31. Syrups should be kept in a cool, dark place, in a well-dried, filled, and well-stoppered bottle. They are kept at a temperature of no more than 25°C. A bottle should be filled, tightly closed, and kept dry. Syrups are self-preservatives. Preservatives such as methylparaben, sodium benzoate, benzoic acid, glycerin, and others are used to prevent bacteria and mould growth.

The world of herbal medications is vast and there is still much to learn about them. It’s time to spread awareness about them all across the world.

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EFFECTS OF VERAPAMIL AND FERULIC ACID AGAINST CHEMICALS INDUCED CONVULSIONS IN ALBINO MICE

Prajakta S. More[1]*, Vitthal J. Chawre[2] and Vivekkumar K. Redasani[3]

[1]Department of Pharmacology, YSPM'S Yashoda Technical Campus, Faculty of Pharmacy, Wadhe Satara India.

[2]Head of Pharmacology, YSPM Yashoda Technical Campus, Wadhe Satara, India.

[3]Principal ,YSPM's Yashoda technical campus, Wadhe Satara, India.

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***Corresponding Author**
Prajakta S. More
Department of Pharmacology, YSPM'S Yashoda Technical Campus, Faculty of Pharmacy, Wadhe Satara India.

ABSTRACT

Background: The currently available antiseizure drugs have a low therapeutic index and provided emerge satisfactory seizure control in only 60-70% of patients. Calcium channel blocker have shown potentials of a useful add-on drug for the available antiepileptic drugs. Role of oxidative stress in epileptogenic process has been supported in various studies. **Objectives:** To study Potentiation effect of verapamil and Ferulic acid against pentylenetetrazole and picrotoxin induced convulsions in mice. **Methods:** For this study, swiss albino mice were used. Effects of verapamil and ferulic acid alone and in combination with diazepam (4mg/kg) were studied. Onset of convulsions and duration of convulsions, percentage protection was considered as the index for antiepileptic activity. **Result:** Verapamil (20mg/kg) produced non significant antiseizure effect and ferulic acid at dose (75mg/kg) reduced the convulsions and myoclonic jerk but verapamil and ferulic acid in combination with diazepam potentiate the antiepileptic effect. **Conclusion:** Verapamil and ferulic acid potentiated the antiepileptic effect of diazepam. Dose of diazepam can be reduced in epileptic patient receiving verapamil and ferulic acid

KEYWORD: Verapamil, Diazepam, Ferulic acid, Picrotoxin induced seizures, Pentylenetetrazole induced seizures.

1. INTRODUCTION

Epilepsy a chronic disorder of heterogeneous symptoms characterized by recurrent seizures,

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of cerebral brain.[1-3] It is the second most common chronic neurological condition observed in worldwide.[4] Seizures that can happens spontaneously and repeatedly are knows as outward signs of epilepsy.[5] Epileptic seizures are characterized by increasing excitability in brain structures (such as within the cortex and subcortical area).[6]

Approximately 1 % of to the world population has epilepsy[7,8] The therapeutic objectives of the treatment of epilepsy is complete seizures control without excessive side effect. Uncontrolled epilepsy can result neuropsychiatric and social impairment, lower quality of life and higher risk of death.[9] Many of the existent ACD produce a host of undesirable side effects including teratogenesis, drowsiness, mental dullness, nausea, ataxia, hematologic changes, hirsutism, weight gain. For these reasons, new ACD are needed to improve seizure control and reduce the side-effect profile (Gasior et al., 1997).[10,11]

The history of hypertension appears to be an independent risk factor for new onset unprovoked seizures.[12] Overwhelming evidence indicate that calcium ions plays an essential role in the pathophysiology of epilepsy. During seizures one can observe a decrease in extracellular calcium concentration prior to onset of seizures activity followed by increase in the intracellular calcium concentration. An important characteristics all CCBs is their ability to inhibit the inward flow of calcium[13] CCBs have several advantages over the existing antiepileptic drugs,such as no effect on hepatic microsomal enzymes ,devoid sedation and wide therapeutic range.[14]

Some conventional antiepileptic drugs induced oxidative stress which limit their clinical condition. Ferulic acid is phenolic phytochemical with antioxidant and neuroprotective properties that prompted to evaluate its therapeutic potential in epilepsy. Which is usually associated with oxidative stress.[15]

2. MATERIALS AND METHODS

2.1 Animals

Swiss Albino mice of body weight 20-30 g were procured from Animal House of Yashoda Technical Campus, Faculty of Pharmacy, satara (Dist-satara) and fed with commercial pellet diet (Hindustan Lever Kolkata, India) and water *ad libitum* were used in this study. All procedures described were reviewed and approved by the IAEC, Yashoda Technical Campus faculty of Pharmacy, Satara. Dist.– Satara (Maharashatra)

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2.2 Drugs and Chemicals

P C Chem provided Picrotoxin and pentylenetetrazole drug, Diazepam (valium tablet) was purchased from Abbott healthcare Pvt, Ltd and Verapamil (Calaptin) was purchased from Abbott Healthcare Pvt, Ltd, Sakshi corporation navi Mumbai provided ferulic acid.

2.3 Inclusion criteria

A majority of mice showed tonic clonic seizure, clonic seizure, myoclonic twitches, hind limb extentions & recovery. Only those rats. showing the convulsive responses were used for experiment.

2.4 Animal care

Swiss albino mice (18-22 g) were selected. Animals were housed under an alternative 12 h light/dark cycle in polypropylene cages with softwood granulate bedding. Three animals were housed in a single cage. Pelleted food and water were made available ad libitum. Animals used in these studies were maintained in facilities fully accredited by the CPCSEA and all experiments were performed under protocols approved by the Institutional Animal Ethics Committee (YSPM/YTC/Pharma /2021-2022/IAEC/003)

2.5 Induction of convulsion by picrotoxin

Picrotoxin (10mg/kg) was administrated and the animals were observed until occurrence of extension -flexion of forelimb and hind limb with falling on back sometimes with spasm of neck muscles (clonic tonic seizures). Latency period of seizure and number of convulsed/ all number of animals in each group were recorded.

2.6 Induction of convulsions by pentylenetetrazole

Pentylenetetrazole (PTZ) –induced seizures in mice is an accepted in-vivo model for the screening of antiepileptic drugs. Seizures are induced by the administration of 80 mg/kg, i.p PTZ and the mice are then observed for a 120 minute period. Mice were administered drugs for days and on experimental day, PTZ 80mg/kg was injected intraperitoneally to mice 45min after vehicles or drugs and 30 min after the standard drug. Immediately after PTZ administration mice were observed.

2.7 Experimental groups

Anticonvulsant studies

2.7.1 Picrotoxin induced convulsion

In toxicant control (n=6), mice were injected with picrotoxin (10mg/kg), Standard group (n=6) mice were injected with Diazepam(4mg/kg) ,Test I -received picrotoxin(10mg/kg),Test II- received standard diazepam(4mg/kg),Test group III–received verapamil (20mg/kg) intraperitoneally; Test group IV– received Ferulic acid (75mg/kg) intraperitoneally, Test group V-received verapamil + Diazepam (20mg/kg+ 2mg/kg), Test group VI- received ferulic acid +Diazepam (75mg/kg+2mg/kg).Thirty minutes after pretreatment,10mg/kg of picrotoxin was administered to each mice. They were then observed for tonic hind limb seizures for 30 min period.

2.7.2 Pentylenetetrazole induced convulsion

Animal were divided into VI groups, (n=6 mice of either sex in one group). Group I received pentylenetetrazole (80mg/kg),group II was allotted for Diazepam (4mg/kg) and Group III received verapamil (20mg/kg) intraperitoneally; Group IV– received Ferulic acid (75mg/kg) intraperitoneally, Group V-received verapamil + Diazepam (20mg/kg+ 2mg/kg), Group VI- received ferulic acid +Diazepam (75mg/kg+2mg/kg). mice were administered drugs for seven days and on experimental day, PTZ 80mg/kg was injected intraperitoneally to mice 45min after vehicles or drugs and 30 min after the standard drug. Immediately after PTZ administration mice were observed for (1) onset of convulsions, (2)incidence (number of mice showing convulsions and (3) mortality for the duration of 30 minutes.

2.7.3 Statistical analysis

The data obtained by the various parameters was statistically evaluated by one way analysis of variance (ANOVA) followed by Dunneet's multiple Comparison Test by Graph pad prism software (GraphPad software inc.., Version 5.0.0). The mean values ± SEM were calculated for each parameter. Level of significances was kept at $p < 0.05$.

3. RESULTS

In Pentylenetetrazole induced convulsions the parameters like onset of convulsions, duration of convulsions and percentage protection were recorded and result obtained in different groups represented in table No 1.

As seen in table No 1, group II (diazepam) showed complete abolition of convulsions, highly

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significant decrease in duration of convulsions, increase in seizure protection.

In group V (verapamil + diazepam) and group VI (ferulic acid + diazepam) showed significant delay onset of convulsions and reduced duration of convulsions and decreased mortality percentage when compared to normal control group ($p < 0.05$)

Table no. 1: Effect of Verapamil and Ferulic acid alone and its combination with diazepam on pentylenetetrazole induced convulsions.

Experimental group	Dose	Onset of convulsions (min)	Duration of convulsions (min)	No of Animals survived	Percentage protection (%)
Group I Toxicant (PTZ)	80 mg/kg i.P	1.08± 0.10	3.10±0.23	0/6	0%
Group II Standard (Diazepam)	4mg/kg i.p	-	-	6/6	100%
Group III (Verapamil)	20mg/kg i.p	2.10±0.1	5.20±0.26	4/6	66%
Group IV (Ferulic acid)	75mg/kg i.p	1.20±0.10	13.10±1.10	2/6	33%
Group V (Diazepam +verapamil)	2mg/kg+20 mg/kg i.p	2.68±0.20	6.10 ±0.32	6/6	100%
Group VI (Diazepam +ferulic acid)	2mg/kg+75 mg/kg i.p	2.10±0.16	7.18±0.31	6/6	100%

All Values expressed as mean ± SEM; n=6 mice in each group, by one -way ANOVA followed by Dunneet's Multiple Comparison Test (Compared with toxical control) $p<0.05$.

In Picrotoxin induced convulsions the parameters like onset of convulsions, duration of convulsions and percentage protection were recorded and result obtained in different groups represented in table No 2.

As seen in table No 2, Group II (diazepam) showed complete abolition of convulsions, highly significant decrease in duration of convulsions, increase in seizure protection.

In group V (verapamil + diazepam) and group VI (Ferulic acid + diazepam) showed significant delay onset of convulsions and reduced duration of convulsions and decrease mortality percentage when compared to normal control group ($p < 0.05$).

Table no. 2: Effect of Verapamil and Ferulic acid Alone and Its combination with diazepam on picrotoxin induced convulsions.

Experimental group	Dose	Onset of convulsions (min)	Duration of convulsions (min)	No of animals survived	Percentage protection
Group I Toxicant (Picrotoxin)	10mg/kg i.p	2.07± 0.10	10.5 ±0.88	0/6	0%
Group II Standard (Diazepam)	4mg/kg i.p	-	-	6/6	100%
Group III (Verapamil)	20mg/kg i.p	12.35±1.10	11.05±0.78	3/6	50%
Group IV (Ferulic acid)	75mg/kg i.p	18.17±1.50	13.73 ±1.01	0/6	0%
Group V (Diazepam +verapamil	2mg/kg+ 20mg/kg i.p	9.10±0.48	8.15±0.42	6/6	100%
Group VI (Diazepam +ferulic acid)	2mg/kg+ 75mg/kg i.p	10.07±0.54	9.34 ±0.31	6/6	100%

All Values expressed as mean ± SEM; n=6 mice in each group, by one -way ANOVA followed by Dunneet's Multiple Comparison Test (Compared with toxical control) $p<0.05$.

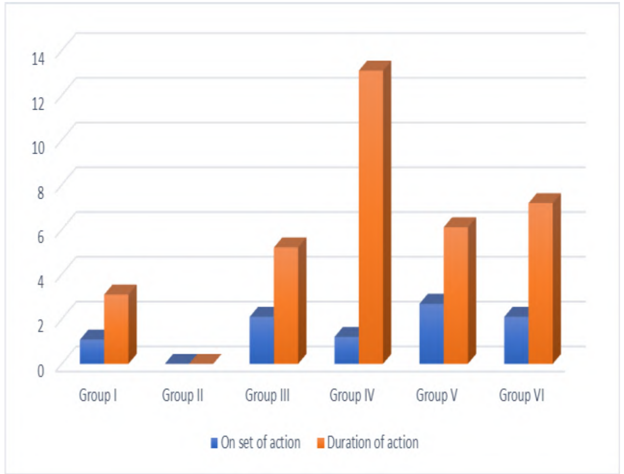

Fig. no. 1: Effect of Verapamil and Ferulic acid Alone and Its combination with diazepam on pentylenetetrazole induced convulsions.

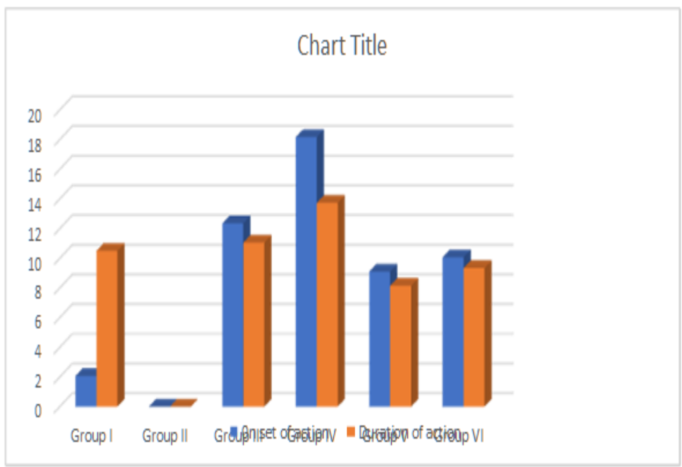

Fig. no. 2: Effect of Verapamil and Ferulic acid Alone and Its combination with diazepam on picrotoxin induced convulsions.

4. DISCUSSION

Epilepsy is characterized by spontaneous recurrent seizures in which electrical activity in particular brain regions becomes over-excitable. Epilepsy is managed mainly with drugs; however, antiepileptic drugs currently in use neither provides a cure nor prevent relapse and are associated with many side effects such as fatigue, allergies, sedation, blood dyscrasias and teratogenesis, changes in mood and memory problems As a result of this, development of new, affordable and accessible pharmacological agents that can overcome these limitations has become a major goal in epilepsy research.[16]

PTZ may be exerting convulsant effect by inhibiting the activity of GABA receptors. GABA is a major inhibitory neurotransmitter in the brain and the inhibition of its neurotransmission promotes seizures.[17]

Antioxidant play an important role in anti seizure activity, it should be reduced the oxidative stress in epilepsy. Epilepsy is one of the most common neurological disorders. However, the Patho physiological mechanisms of epilepsy are not yet fully understood. Recent years have focused on the role of oxidative stress in seizures. There is emerging evidence that focuses on the role of oxidative stress and mitochondrial dysfunction both as a consequence and a cause of epileptic seizure. Experimental seizures are known to be associated with a massive release of reactive oxygen species.[18]

Ferulic acid [(E)-3-(4 hydroxy-3-methoxy-phenyl)prop-2-enoic acid) is an active phenolic constituent of many plant species which has shown a wide range of pharmacological

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properties including the effects against the inflammation, oxidative stress, cancer, hepatotoxicity, diabetes, thrombosis. Moreover, isopentyl ferulate (an ester derivative of ferulic acid) has shown. anticonvulsant effect in mice.[19]

During seizures one can observe a decrease in the extracellular calcium concentrations prior to onset of seizure activity followed by an increase in the intracellular calcium concentrations. An important characteristic of all CCBs is their ability to inhibit the inward flow of calcium ions. CCBs depress the epileptic depolarization of neurons has shown the presence of specific binding sites of CCBs that enable them to cross the blood brain barrier. This gives important evidence for the presence of central effects of CCB.[20,21]

In our investigation, verapamil and ferulic acid in combination with diazepam was evaluated in behavioral study that gives good indication of protection and reduced latency of convulsions induced by pentylenetetrazole and picrotoxin. Verapamil and ferulic acid alone shows anticonvulsant effect also but verapamil and ferulic acid in combination with diazepam shows more significant effect than alone drugs treatment. Verapamil and ferulic acid potentiated antiepileptic effects of diazepam.

5. CONCLUSION

It was concluded that verapamil alone at the dose 75mg/kg i.p could significantly delayed onset of convulsion and increased percentage protection of animals from death. Ferulic acid did not possess anticonvulsant activity.

Diazepam at the dose 4mg/kg significantly abolish onset of convulsion and duration of convulsion at compare to toxicant control animals.

Verapamil and ferulic acid potentiated the antiepileptic effect of diazepam. Dose of diazepam can be reduced in epileptic patient receiving verapamil and ferulic acid. However it need further confirmation to establish clinical utility of verapamil and ferulic acid.

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POTENTIATION OF EFFECTS OF PROPRANOLOL AND HEPARIN BY ANTIOXIDANT IN ADRENALINE INDUCED MYOCARDIAL INFARCTION IN RATS

Manasvi P. Patil*, Vitthal J. Chaware and Vivekkumar K. Redasani

Department of Pharmacology, YSPM's Yashoda Technical Campus Wadhe, Satara, India.

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***Corresponding Author**

Manasvi P. Patil

Department of Pharmacology, YSPM's Yashoda Technical Campus Wadhe, Satara, India.

ABSTRACT

Background: Myocardial Infarction also known as heart attack is still a major cause of morbidity and mortality around the world. Vitamin C is a powerful antioxidant that can strengthen body's natural defenses and propranolol and heparin are the drugs which affect the cardiovascular system. So there is need to see if Propranolol, heparin, vitamin C and combinations of vitamin C with low doses of propranolol and heparin can show cardioprotection against adrenaline induced myocardial infraction (MI) in rats. **Objective:** To study cardioprotective effect of Vitamin C and its combination with low dose of propranolol and heparin against adrenaline induced myocardial infarction (MI) in rats. **Materials and Methods:** Rats were divided randomly into seven groups (6 rats each); Group I-Normal control; Group II-Toxicant control (adrenaline treated group); Group III-rats were pre-treated with propranolol (10 mg/kg p.o) for 10 days (from day 12 to day 21); Group IV-rats were pre-treated with heparin (500 units/kg) for 2 days (on 20th and 21th day); Group V-rats were pretreated with vitamin C (40 mg/kg i.p) for 21 days; Group VI-rats were pretreated with vitamin C (40 mg/kg) and propranolol (5 mg/kg) for 21 days and from 12 to 21 days respectively; Group VII-rats were pretreated with vitamin C (40 mg/kg) and heparin (250 units/kg) for 21 days and on 20th -21th day respectively. The rat model of myocardial infarction was produced by injecting adrenaline (2 mg/kg b.w.) subcutaneously into all animals (except the control) twice at a 24-hour interval on days 20th and 21th day of the experiment. The cardiac markers tests were done at the conclusion of the trial. **Results:** Treatment with Adrenaline showed significantly increased in biochemical parameters (CK-MB, LDH, SGOT, Troponin-I). Prior treatment with propranolol, heparin, vitamin C and its combination with low doses of propranolol and heparin showed significant

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alteration in these parameters. **Conclusion:** The results of present study highlights that propranolol, heparin and vitamin C when given alone showed cardioprotection but combination of vitamin C with Propranolol and Heparin with low doses has showed better cardioprotection based on the alterations of cardiac biomarkers.

KEYWORDS: Myocardial infarction, Adrenaline, Oxidative stress, Inflammation, Propranolol, Heparin, Vitamin C, Cardiac biomarkers.

1. INTRODUCTION

Cardiovascular Diseases CVDs, often known as silent killers, are the largest cause of disease burden and mortality around the world. Annual CVD deaths in India are expected to increase from 2.26 million in 1990 to 4.77 million in 2020, accounting for 29 percent of all deaths worldwide (17.9 million deaths), and by 2030, more than 23.3 million people would die from CVDs (WHO).[1] Coronary heart disease, cerebrovascular disease, congestive heart failure, and other heart and blood vessel illnesses are all classified as CVDs. Heart attacks account for more than four out of every five CVD deaths.

Myocardial infarction, often known as a heart attack, is a life-threatening disorder in which blood flow to the heart's coronary artery slows or ceases, causing damage to the heart muscle. It is the irreversible destruction of heart muscle caused by a lack of oxygen for an extended period of time. As a result, there is a mismatch between coronary blood supply and myocardial demand. It's linked to an inflammatory response and a change in the extracellular matrix as a result of free radical release.[2] Prolonged MI causes ischemia and cardiac cell death.[3] It has been well characterized that oxidative stress and inflammation are the main pathophysiological process involved in Myocardial Infarction.[4] The severity of cardiac lesions can be influenced by the inflammatory process. Anti-inflammatory medications may help to minimise the size of ischemic lesions in myocardial ischemia. Antioxidant therapy can also have cardioprotective effects by lowering oxidative stress during myocardial ischemia and reperfusion damage. The enzymes most widely used in the detection of MI are Troponin I, Creatine kinase-MB(CK-MB), Lactate dehydrogenase(LDH).[5]

Adrenaline, also known as epinephrine, is a stress hormone produced by the adrenal glands and released into the bloodstream. It is a component of the "fight or flight" reaction. Catecholamine is a naturally occurring substance. It also has medicinal uses in the treatment of cardiac arrest, allergic responses, and asthma, among other things. However, at doses

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beyond physiological levels, it has been shown to increase the generation of reactive oxygen species (ROS) and tissue damage caused by reactive nitrogen species (RNS). Adrenaline-induced MI in rats is regarded as a reliable experimental model for studying medication cardioprotective effects. Adrenaline has been discovered to cause MI by promoting lipid peroxidation, which causes cellular antioxidants to be depleted.[6]

Vitamin C is a water-soluble antioxidant and cofactor for enzymes in both plants and animals.[7] It is a potent antioxidant that helps the body's natural defences against oxidative stress mediated by reactive oxygen species (ROS) by shielding cells from free radicals, which are damaging chemicals. Consuming extra vitamin C raises your blood antioxidant level, which aids the body's natural defences in fighting inflammation, according to studies.[8] Futhermore, vitamin C intake from food or suppliments can increase the bioavailability of iron by improving the absorption of the non-heme iron.

Propranolol is a nonselective beta-adrenoreceptor antagonist. It has a number of pharmacological properties that may be useful in MI and support its usage. It exerts its response by competitively blocking beta-1 and beta-2 adrenergic stimulation in the heart, which is typically induced by adrenaline.[9] It exerts its effects primarily by blocking the action of the endogenous catecholamines, epinephrine and norepinephrine.[10] antagonizes the action and relieve stress on the muscle. It has the property to decrease the workload of heart by slowing the heart rate and force of contraction and also decrease the demand of oxygen.

Heparin is an anticoagulant which is known as blood thinner, a chemical substance that prevent or reduce coagulation of blood prolonging the clotting time. It interacts with the naturally occuring plasma protein, Antithrombin III, to induce conformational change. It inhibit Factor Xa and thrombin (Factor II a). It also prevent the formation of a stable fibrin clot by inhibiting the activation of the fibrin stabilizing factor.[11] It blocks the activity of coagulation factor and is used for reinfarction and thromboembolism and can lead to reduction in dead.

In this study, we assess the cardioprotective effects of propranolol, heparin, vitamin C and its combination with low doses of propranolol and heparin in rats with adrenaline-induced myocardial infarction. Since propranolol and heparin are used to treat MI, but the study of low doses of these drugs has not been made and if this drugs are combined with antioxidant then enhancement in immune system will take place and can show advanced effect which can

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enhance immune system and improve its function. This research also sheds light on how metabolic changes affect therapy efficacy.

2. MATERIALS AND METHODS

2.1. Experimental animals

The experiments were conducted according to ethical guidelines as approved, Wistar Albino rats were used in the present study. These rats were procured from registered breeder and was acquainted in the quarantine area for one week. After acquaintation, animals were transferred to the standard laboratory conditions of 22 ± 2ºC temperature, 50 ± 15% of relative humidity, 12 hr dark/12 hr light cycle and the animals received free access to pellet diet & water provided *ad libitum*. The study protocol was presented to the IAEC and was approved.

2.2. Drugs and Chemicals

Adrenaline was purchased from Aqua fine injecta pvt. ltd. pune, Propranolol and vitamin C were purchased from Sigma-Aldrich Chemical Co. (st. Louis, USA), Heparin from Pfizer Labs division of Pfizer Inc NY, NY 10017, CK-MB, LDH, SGOT and Troponin kits was purchased from Meril Diagnostics Pvt. Ltd, Gujarat, India.

2.3. Induction of experimental MI

Adrenaline (2 mg/kg body weight) was subcutaneously injected into all groups of rats except normal control group for 2 consecutive days at an interval of 24 h to induce experimental myocardial infarction.

2.4. Experimental protocol

A total of 42 wistar albino rats were used for this study. After acclimatization, they were randomly divided into seven groups, consisting of six rats per group.

2.4.1. Group I (Normal control)

Served as untreated normal control rats and received distilled water (1 ml p.o).

2.4.2. Group II (Toxicant control)

Animals received Adrenaline (2 mg/kg body weight s.c) on 20th and 21th day.

2.4.3. Group III (Propranolol + Adrenaline)

Animals received Propranolol (10mg /kg body weight p.o) for 10 days (from 12 to 21 days) and challenged with adrenaline (2 mg/kg, s.c) on 20th and 21th day.

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2.4.4. Group IV (Heparin + Adrenaline)

Animals received heparin (500 units/kg body weight s.c) on 20th and 21th day and challenged with adrenaline (2 mg/kg, s.c) on 20th and 21th day.

2.4.5. Group V (Vitamin C + Adrenaline)

Animals received vitamin C (40 mg/kg body weight i.p) for 21 days from and challenged with adrenaline (2 mg/kg, s.c) on 20th and 21th day.

2.4.6. Group VI (Vitamin C + Propranolol + Adrenaline)

Animals received vitamin C (40 mg/kg body weight i.p) for 21 days with Propranolol (5 mg/kg body weight p.o) for 10 days from 12th -21th and challenged with adrenaline (2 mg/kg, s.c) on 20th and 21th day.

2.4.7. Group VII (Vitamin C + Heparin + Adrenaline)

Animals received vitamin C (40 mg/kg body weight i.p) for 21 days with heparin (250 units/kg body weight s.c) on 20th and 21th day and challenged with adrenaline (2 mg/kg, s.c) on 20th and 21th day.

2.5. Biochemical estimation

The blood samples were taken from the retro-orbital plexus and centrifuged to separate them. The serum of numerous experimental animals was collected and utilised to perform various biochemical analyses.

The activities of serum creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), Serum glutamic oxaloacetic Transaminase (SGOT) and Troponin-I were estimated using commercially available kits as per instructions.

2.6. Statistical analysis

Data were expressed as mean $\pm$ S.E.M (six rats per group). Groups of data were compared by one-way analysis of variance (ANOVA) followed by Dunnett's t-test. Values $p < 0.05$ were consider statistically significant. Statistical analysis was carried out using Graph Pad Prism 5.0 software (Graph Pad Software, San Diego, California, USA).

3. RESULTS

3.1. Effects of Vitamin C and its combination with low doses of Propranolol and Heparin against adrenaline induced MI in rats

The effects of Propranolol, Heparin, Vitamin C and its combination with propranolol and heparin on Cardiac biomarkers are seen in the table No:1. Rats treated with adrenaline (Group II) showed a significant increase in the level of serum LDH, CK-MB, SGOT and Troponin-I (Positive) as compared to the normal control (Group I). Pre-treatment with propranolol (10 mg/kg), heparin (500 units/kg) and vitamin C (40 mg/kg) when given alone reduced the elevated level of the cardiac markers induced by adrenaline. Prominent inhibition effect on cardiac markers was observed when animals were treated by combination of vitamin C with low dose of propranolol and heparin.

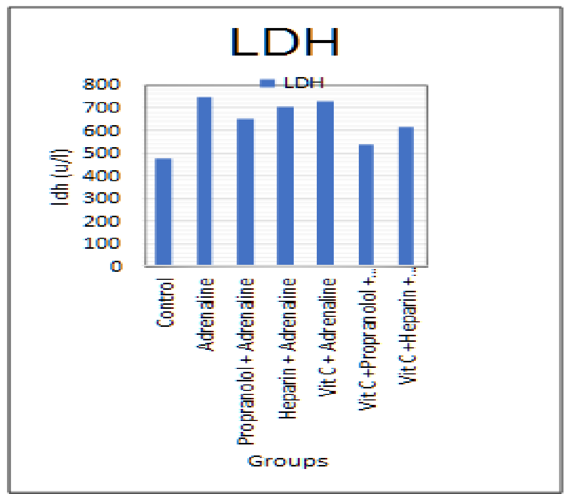
Table no. 1: Effects of vitamin C and its combination with low doses of propranolol and heparin against adrenaline induced MI in rats.

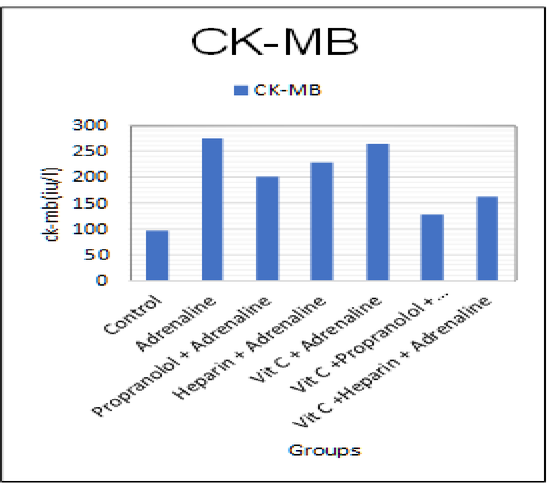
Groups	CK-MB(IU/L)	LDH(U/L)	SGOT(IU/L)	Troponin-I
Control	96.44±3.05	477.22±16.35	56.83±02.33	Negative
Adrenaline	273.77±12.09	749.55±33.13	219.44±07.33	Positive
Propranolol + Adrenaline	201.36±10.33	649.41±22.63	155.13±10.33	Negative
Heparin + Adrenaline	228.41±11.88	705.31±35.33	187.35±09.50	Negative
Vitamin C + Adrenaline	264.25±12.48	731.58±11.27	201.21±12.11	Negative
Vitamin C +Propranolol + Adrenaline	129.12±05.33	537.13±16.33	126.57±06.33	Negative
Vitamin C +Heparin + Adrenaline	162.51±8.33	613.24±25.13	141.29±10.48	Negative

Data are expressed as mean ±S.E.M (n= 6 animals in each group). Statistical analysis are carried out by one-way ANOVA followed by Dunnett's t-test.

Significance difference from normal group at $p < 0.05$.

Significance difference from control (MI) group at $p < 0.05$.





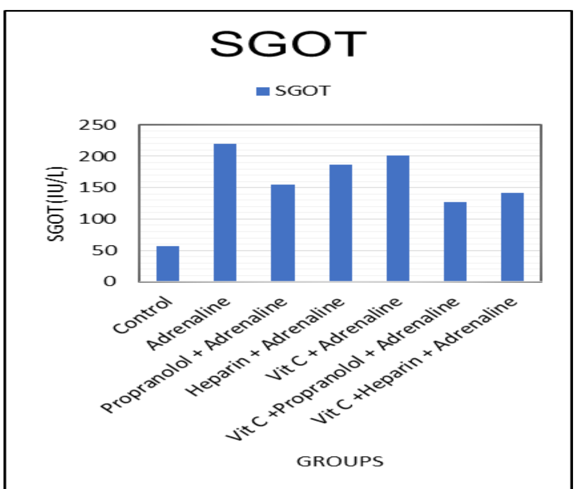


Fig. 1: Graphical representation of effects of Vitamin C and its combination with low doses of propranolol and heparin against adrenaline induced MI in rats.

4. DISCUSSION

In 2021, myocardial infarctions were on the rise, and there was no age limit. This year, young, healthy, fit persons with no medical history have suffered heart attacks, with some cases even resulting in death, contributing to an increase in mortality. The pandemic of COVID had also contributed in the increasing cases of heart attacks.

For a long time, myocardial cell protection and cell necrosis prevention have been therapeutic targets. Because present treatments have a limited influence on survival and annual expenditures, new therapeutics are needed to treat myocardial infarction.

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The purpose of this investigation was to evaluate the potential cardioprotective role of propranolol, heparin, vitamin C and combination of vitamin C with low doses of propranolol and heparin in adrenaline induced MI in rats.

In this study, adrenaline was administered subcutaneously at a dose of 2 mg/kg for two days, resulting in a significant increase in blood levels of cardiac enzymes CK-MB, LDH, SGOT, and Troponin-I. This demonstrates that adrenaline injection causes myocardial injury and cardiac enzyme leakage into the circulation. This can take place due to increased in lipid peroxidation, inflammation or apoptosis.[12] Due to lipid peroxidation oxidative stress generates which can lead to the injury or damage to the cells which leads to the increase in the cardiac biomarkers. Our results supports the hypothesis that adrenaline has got the ability to produce free radicals and has caused myocardial infarctions.

Damage to the cell membranes, by production of large number of free radicals and ROS, generation of lipid peroxides, and lowering of antioxidative defense lines are major outcomes of MI.[13]

Since, ROS is important in the pathogenesis of MI, antioxidants and their combinations in the treatment of MI were the focus of research.

Pre-treatment with propranolol, heparin, vitamin C and its combination with low doses of propranolol and heparin has been observed. It was found that the dugs shows cardioprotective effects.

In recent years, long-term prevention of CVD is associated with consumption of fresh fruits, vegetables or plants rich in antioxidant. As a result there has been considerable interest in antioxidants.[14]

Vitamin C is a powerful dietary antioxidant. It is widely known vitamin in different kinds of fruits and is available as a supplement.[15] Antioxidants constitute the foremost defense system that limits the toxicity associated with free radicals.[16] It act by reducing the reactive oxygen species and leads in decrease in lipid peroxidation which is the main cause of depilation of cellular antioxidant and which leads to damage of cells and inflammation which leads to MI. Vitamin C act against it and showed mild effect by decreasing the levels of CK-MB, LDH, SGOT and Troponin-I helps in reducing the impact caused due to adrenaline.

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Propranolol antagonizes the effect of adrenaline by lowering heart rate[17] and reducing the lipid peroxidation as it decreases the demand for oxygen, it also relaxes the blood vessels and improve blood flow with decrease in blood pressure therefore possesses the cardioprotective effect.

As the blood gets clots, when the tissue gets damaged due to the generation of oxidative stress the use of heparin helped in the anticoagulation process by inhibiting the enzymes due to which the clots are reduced. It also attenuates the rate of rise in blood pressure and prevents severe fibrinoid vascular lesions and thus shows cardioprotection.[18]

So, as the drugs showed positive effect there was need to see if the low doses of these drugs shows some advanced effect if combined with the antioxidant.

In this study, when the low doses of propranolol and heparin was combined with vitamin C, the combination with propranolol showed more advanced effect on oxidative stress which lead to lipid peroxidation due to its strong antioxidant property due to which the level of cardiac biomarkers were decreased as compared to that of the adrenaline treated animals (Toxicant group).

Similarly, when it was combined with heparin it showed advanced cardioprotection and lead to decreased inflammation, oxidative stress and blood clots which were observed by alteration of parameters which were caused by adrenaline induced MI in rats.

5. CONCLUSIONS

It can be concluded that propranolol, heparin and vitamin C when given alone improved adrenaline induced abnormal changes in the biomarkers and showed cardioprotective effect in rats after being exposed to adrenaline but the combined administration of vitamin C with low dose of propranolol and heparin produce a potentiation effect and showed better results possibly via reducing cardiac biomarkers associated with oxidative stress, inflammation, and apoptosis. More research is needed to determine the exact molecular pathways involved in the cardioprotective impact of medicines in MI rats.

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EVALUATION OF NEPHRO-PROTECTIVE EFFECT OF DPP4 INHIBITOR AND ANTIOXIDANT AGAINST GENTAMYCIN INDUCED NEPHROTOXICITY IN ALBINO RATS

Shamal J. Kadam[1]*, Vitthal J. Chaware[2] and Vivekkumar K. Redasani[3]

[1]Department of Pharmacology, YSPM's Yashoda Technical Campus Wadhe, Satara, India.

[2]Professor, YSPM's Yashoda Technical Campus Wadhe, Satara, India.

[3]Principal, YSPM's Yashoda Technical Campus Wadhe, Satara, India.

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***Corresponding Author**

Ms. Shamal J. Kadam

Department of Pharmacology, YSPM's Yashoda Technical Campus Wadhe, Satara, India.

ABSTRACT

Background:- Nephrotoxicity is a global health challenge of vast proportion around the world. Recent studies demonstrated the reno-protective effects of two dipeptidyl peptidase-4 (DPP-4) inhibitors, saxagliptin and linagliptin, against gentamycin-induced renal injury. However, none of these studies investigated the combination of DPP 4 inhibitor and antioxidant. This prompted us to test this hypothesis and to assess, for the first time, the potential reno-protective effect of DPP-4 inhibitor and antioxidant. **Objective:-** This study aimed to investigate the potential protective effect of DPP-4 inhibitor and antioxidant on gentamycin-induced nephrotoxicity. **Method:-** Nephrotoxicity was induced in the rats with Gentamycin (100mg/kg). All animals except normal control were intraperitoneally administered with gentamycin at a dose of 100mg/kg once daily for 10 days. Respective treatment were started from day 2nd till day 14th (2 weeks). On the 15th day, blood samples were collected through retro-orbital plexus under anesthesia. Serum was separated to measure creatinine, BUN, uric acid, proteins & MDA (Malondialdehyde). Body weight were also recorded. **Result:-** Administration of combination of DDP-4 inhibitor and antioxidant ameliorated gentamycin induced renal injury and restored renal functional, oxidative, inflammatory, apoptotic & histopathological changes. **Coclusion:-** These findings suggest that combination of DDP-4 inhibitor and antioxidant treatment attenuate renal dysfunction and structural damage through the reduction of oxidative stress, mitochondrial dysfuction and apoptosis in the kidney.

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Yashoda Technical Campus
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KEYWORDS: Nephrotoxicity, Gentamycin, DDP-4 inhibitor, antioxidant, Renal Biomarkers.

1. INTRODUCTION

Nephrotoxicity can be defined as the adverse effect of substances on renal function.[1,3] These substances can include molds and fungi, cancer therapeutics such as cisplatin, antibiotics such as aminoglycosides, metals such as mercury, arsenic and lead, and drugs of abuse such as cocaine.[2,3] Due to relatively large blood flow (20 % of stroke volume) and the ability to extract and concentrate hydrosoluble toxic molecules, the kidney is prone to drug induced damage. The experimental data point to the fact that drug induced nephrotoxicity includes multiple mechanisms that can be classified as vascular, glomerular and tubular. The kidney damage is usually a consequence of tubular obsruction caused by cell swelling or debris deposition.[4,6] Toxic substances can damage various cell types in kidney. The most studied effect is necrosis of tubular epithelial cells.[5,6] One indication of nephrotoxicity is a change in renal function as assessed by the glomerular filtration rate (GFR), blood urea nitrogen (BUN), serum creatinine (sCr), or urine output.[2,3]

Aminoglycoside antibiotics are commonly used for the treatment of severe gram negative bacterial infections. Despite their beneficial effects, aminoglycosides have considerable nephrotoxic side effects.[7,9] The most widely used drug in this category is gentamycin.[8,9] Nephrotoxicity remains the major side effect hindering the clinical use of the aminoglycoside, gentamycin.[10,14] A small fraction of the administered dose preferentially accumulates in the proximal tubules, inducing oxidative stress, apoptosis, necrosis of renal cells and eventually acute renal injury and damage.[11,14] Therefore, many approaches were adopted to mitigate the progression of the renal injury as once-a-day administration regimen.[10,14] Neprotoxicity induced by GEN is a complex phenomenon characterised by an increase in blood urea nitrogen (BUN) and serum creatinine (Cr) concentration, and severe proximal renal tubular necrosis followed by deterioration and renal failure.[15,9]

Incretins; glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), are gut hormones secreted from the intestinal cells in response to food intake. Once reaching the circulation, they potentiate the glucose dependent insulin secretion from pancreatic cells and inhibit glucagon secretion.[12,14] The incretin effect is significantly decreased in patients with type 2 diabetes (T2D) and contributes to impaired insulin secretion

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and chronic hyperglycemia. GLP-1 and GIP are rapidly inactivated by the dipeptidyl peptidase-4 (DPP-4).[13,14]

Glutathione (GSH) is the most abundant nonprotein thiol and has many functions in vivo. The major role of GSH is the maintenance of cellular redox balance. It plays a role as a substrate of glutathione peroxidase, an antioxidative enzyme that scavenges various peroxides.[16] The physiological role of GSH as an antioxidant has been described and substantiated in studies of numerous disorders reflecting the increased oxidation is a result of abnormal GSH metabolism.[17,18] GSH is thought to be an important factor in cellular function and defense against oxidative stress, such as radiation and drug resistance. Many reports have demonstrated that GSH acts as an endogenous antioxidant.[19,20]

However, there have been no prior studies demonstrating a protective effect of sitagliptin (DPP-4 inhibitor) & GSH against the gentamycin induced nephrotoxicity. In this study, we demonstrated for the first time that combination of sitagilptin and GSH suppresses oxidative stress in vivo, and the impairment of renal function.

2. MATERIALS AND METHODS

2.1. Animals:- The study was approved by the Institutional ethics committee. 30 Wistar Albino rats (200-250gm) were selected for present study. The animals were housed at room temperature (22-28 ^{o}C) 12 hr dark and light cycle and given standard laboratory feed and water *ad - libitum*. Experiments were conducted in strict accordance with CPCSEA guidelines.

2.2. Drugs and Chemicals:- Gentamycin sulfate ampoules was obtained from Abbott (Mumbai), Sitagliptin was purchased from Sun Pharmaceutical Industries Ltd., Glutathione were purchased from HK Vitals, Chloroform were purchased from commercial vendors.

2.3. Experimental protocol:- Animals were divided into five groups, six animals each. Control Group received 2 ml/kg/day vehicle orally. Toxicant Control Group received gentamycin (100 mg/kg/day) intraperitoneally. Test I Group received sitagliptin (30 mg/kg/day) by oral gavage simultaneously with gentamycin. Test II Group received glutathione (300 mg/kg/day) intraperitoneally with gentamycin. Test III Group received combination of sitagliptin (30 mg/kg/day) and glutathione (300 mg/kg/day) with

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gentamycin. Treatment continued for 14 days. Respective treatment were started from day 2nd till day 14th (2 weeks).

2.4. Sample collection:- On the 14th day, after the last gentamicin injection was applied, rats were placed in individual metabolic cages. On 15th day blood samples were collected through retro-orbital plexus under anaesthesia. Serum was separated to measure creatinine, BUN, urea, uric acid, MDA.

2.5. Biochemical determination:- The determination of serum creatinine (CliniQuant-FSR, Jaffe,s Method, Initial Rate, Creatinine assay kit), serum uric acid (CliniQuant-FSR, Uricase – Tinder, End Point, Uric acid assay kit), blood urea and blood urea nitrogen (CliniQuant-FSR, Urease – GLDH, Fixed Time, Urea (BUN) kit), MDA (abbexa MDA ELISA Kit) was done as instructed by manufacturer.

2.6. Data Analysis and Statistics:- Data were expressed as means ± SD. Statistical significance was tested with the one-way analysis of variance (ANOVA) followed by Bonferroni's Test as a post hoc test using GraphPad Prism version 5.00. Probability < 0.05 was considered significant.

3. RESULTS

Effect of sitagliptin, glutathione & combination of both on body weight

After two weeks of treatment, rats showed significant change in body weight because of inflammation of kidney. Final weight of rats in positive control group was significantly higher than initial weight of rats as compared to control group, whereas test group I, II & III showed reduction in gain of weight of rats than that of toxicant control group.

Effect of sitagliptin, Glutathione & Combination of both on renal biomarkers (Blood Urea, Blood Urea Nitrogen, Serum Creatinine, Serum Uric acid, MDA)

As shown in Table No. 1, Group II (Toxicant control) were injected with gentamycin (100 mg/kg). It showed significantly elevated levels of urea, BUN, creatinine, uric acid, MDA as compared to the control group.

Group III (Test I) were treated with sitagiptin (30 mg/kg). It showed slightly reduction in the elevated levels of urea, BUN, creatinine, uric acid & MDA as compared to toxicant control group.

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Group IV (Test II) were treated with glutathione (300 mg/kg). It showed slightly readuction in the elevated levels of urea, BUN, creatinine, uric acid & MDA as compared to toxicant control group.

Group V (Test III) were treated with combination of sitagliptin (30 mg/kg) & glutathione (300 mg/kg). It showed significant reduction in the elevated levels of urea, BUN, creatinine, uric acid & MDA as compared to toxicant control group. There were no statistically difference between Group I and Group IV.

Effect of sitagliptin, glutathione & combination of both on serum proteins

There is no significant difference in the levels of serum proteins (albumin, globulin) in all gropus.

Table 1: Effect of the Test I, II & III group on body weight, Renal Biomarkers & Serum protein.

Parameter	Group I (Control)	Group II (Toxicant control)	Group III (Test I)	Group IV (Test II)	Group V (Test III)
Body Weight					
Initial Body Weight (gm)	223 ± 0.86	227* ± 0.71	234** ± 0.49	238** ± 0.32	231** ± 0.56
Final Body Weight (gm)	234 ± 0.61	256* ± 0.94	250** ± 0.72	249** ± 0.65	246** ± 0.69
Renal Biomarkers					
Blood Urea (mg/dl)	33.2 ± 0.94	179.9* ± 1.81	32.6** ± 0.87	34.7** ± 0.63	31.5** ± 0.79
Blood Urea Nitrogen (mg/dl)	15.5 ± 0.48	84.01* ±1.08	17.8** ± 0.37	16.5** ± 0.55	14.10** ± 0.61
Serum Creatinine (mg/dl)	0.50 ± 0.02	2.56* ± 0.05	0.62** ± 0.03	0.58** ± 0.04	0.55** ± 0.02
Serum Uric Acid (mg/dl)	4.7 ± 0.13	8.7* ± 0.37	5.1** ± 0.12	4.9** ± 0.15	4.6** ± 0.20
MDA Levels (μmol/L)	7.1 ± 0.37	11.4 ±0. 40	7.9 ±0. 39	7.8 ±0. 23	7.5 ±0. 18
Serum Proteins					
Serum Albumin (g/dl)	3.6 ± 0.14	3.4 ± 0.45	3.7 ± 0.96	3.8 ± 0.56	3.5 ± 0.35
Serum Globulin (g/dl)	-3.6 ± 0.25	-3.4 ± 0.52	-3.7 ± 0.38	-3.8 ± 0.69	-3.5 ± 0.58
A/G Ratio	3.6 : 1 ± 0.46	3.4 : 1 ± 0.71	3.7 : 1 ± 0.27	3.8 : 1 ± 0.21	3.5 : 1 ± 0.45

Data represent means ± SD of 6 rats in each group. *$p < 0.05$ compared with control group. **$p < 0.05$ compared with gentamycin group.

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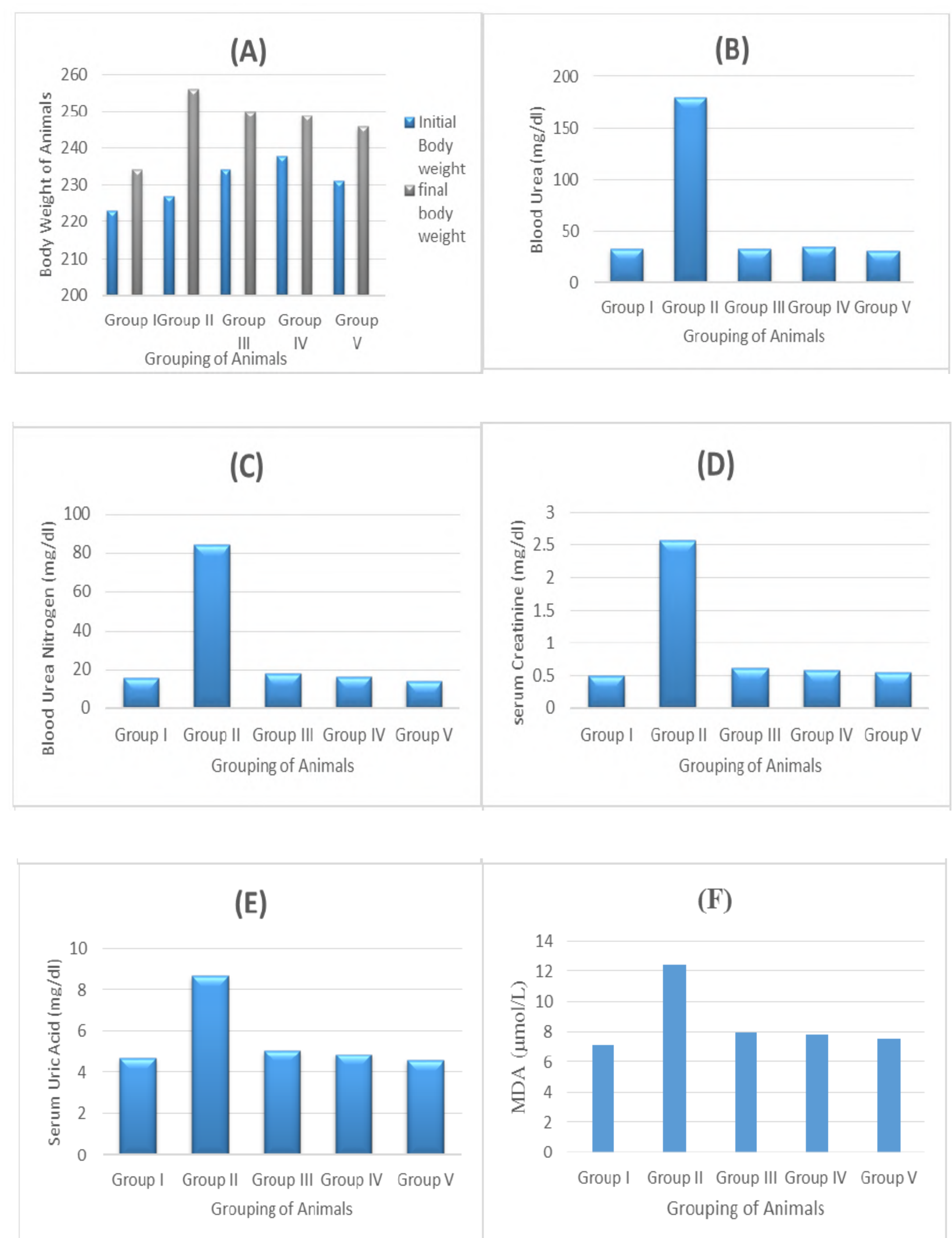

Fig. 1: Effect of sitagliptin, glutathione, combination of sitagliptin and glutathione on gentamycin – induced changes in renal biomarkers. A) Body weight B) Blood urea C) Blood urea nitrogen D) Serum creatinine E) Serum uric acid F) Malondialdehyde. Data represent means ± SD of 6 rats in each group.

4. DISCUSSION

Iatrogenic renal failure is commonly seen as a complication of many therapeutic agents. This can be likely explained by the capability of kidney to extract and concentrate toxic substances

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and its high share of cardiac output. Thus, it is well-documented to be a target for many toxic xenobiotics.[21]

Nephrotoxicity as a side effects of all aminoglycosides, especially Gentamycin, limits its therapeutic use.[22] Aminoglycosides are not metabolized and are essentially eliminated by glomerular filtration. About 10% of the intravenously administered dose is accumulated in the kidney.[23] Gentamycin is largely accumulated in lysosomes, the Golgi and endoplasmic reticulum.[24,25] The serious effect occurs when the concentration of gentamycin inside the previously mentioned organelles exceeds a threshold followed by subsequent destabilization of their membranes with accumulation then release of gentamicin to cytosol.[26,27] Thus, the gentamycin in the cytosol will act on mitochondria and provoke the mitochondrial pathway of inducing oxidative stress, apoptosis and diminish the ATP.[28,29] Gentamycin induces similar morphological alterations in kidneys of both humans and experimental animal.[30] Gentamycin administration produced a elevation of kidney injury markers exhibited as a significant increase of serum BUN and creatinine levels.

In the present study, we aimed to investigate the effect of Sitagliptin and Glutathione against gentamycin-induced nephrotoxicity, hoping to achieve a new therapeutic approach that can protect or reverse gentamycin-induced nephrotoxicity. Sitagliptin significantly counteracted the nephrotoxic effects of gentamicin and retained all the injury markers near the normal levels.[31] Treatment with GM produces oxidative stress in tubular cells, both in vivo in rats[32] and in cultured tubular cells.[33] This oxidative stress is likely to be mediated by hydroxyl radicals, hydrogen peroxide and by superoxide anions[34,35] from mitochondrial origin.[36] GM directly increases the production of mitochondrial ROS from the respiratory chain.[28] The deleterious effect of overproduction of ROS and the process of lipid peroxidation, respectively, damage the protein molecules and degrade the membrane-bound phospholipids.[37] The decreased antioxidant activity in GM-induced nephrotoxicity can be explained by an increase in the generation of free radicals. This is followed by subsequent depletion of antioxidant enzymes during the process of counteracting oxidative stress.[38] Sitagliptin significantly ameliorated all these changes.[31]

Apoptosis contributes in the pathological process of different renal diseases and drug-induced nephrotoxicity.[39,40] About 20% of patients receiving GM treatment could be complicated by acute renal failure with evidence of acute tubular necrosis[33] Experimental studies with GM revealed signs of apoptosis.[4] Earlier studies have pointed that attenuating of apoptosis

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suppresses renal injury which focus on the importance of inhibition of apoptosis as a critical clinical target in renal diseases.[42,43] The intrinsic pathway of apoptosis is found to be initiated by mitochondrial dysfunction.[28] Briefly, GM promotes bax aggregation and translocation to the mitochondria, causing activation of caspase-9, which then activates caspase-3. These events lead to a loss of mitochondrial membrane potential and initiate apoptotic process.[39] Sitagliptin prevented renal tubular apoptosis induced by GM exhibited by a significant decrease in the number of positive brownish caspase-3 and bax immunoreactive cells in kidney sections. These results were supported with the previous reports confirming the anti-apoptotic effects of Sita.[44,45]

Protective roles for antioxidants in genral against free radicals have been demonstrated in a num. ber of in vitro and in vivo experiments. Among the species acting as scanegers, GSH's importance has been widely stressed, depletion of tissue GSH causing hypersusceptibility to some toxic chemicals and radication. Renal function, as indicated by glomerular filtration, etc., is also effected by depletion of GSH.[46] In mammalian cells and tissues, GSH is the most abundant nonprotein thiol; it is usually present in millimolar concentrations.[47,48] As the key intracellular antioxidant, GSH reacts with electrophilic compounds and serves as a reductant for eliminating hydrogen peroxide and lipid hydroperoxides.[49] The main function of exogenous GSH is to suppress lipid peroxidation, which occurs in the plasma membrane and damages the membrane's structure and permeability.[50]

5. CONCLUSION

The present study suggest that sitagliptin alone has renal beneficial effect & that it may serve as an adjuvant to reduce gentamycin induced renal injury in rats. This also suggest that glutathione alone acts as a pontetiate scavenger of free radicals to prevent the toxic effect of gentamycin. But the present study mainly suggest that the combination of boh sitagliptin and glutathione has a more nephropotective potential against gentamycin induced nephrotoxicity. This may be ascribed to their antioxidant, antimitochondrial dysfunction and anti – apoptotic effects. Nonetheless, further studies are needed to investigate different doses of the combination against gentamycin induced nephrotoxicity before safe application in humans.

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PHARMACOLOGICAL EVALUATION OF ANTIDEPRESSANT-LIKE EFFECT OF VITAMIN E AND ITS COMBINATION WITH AMITRIPTYLINE: AN ACUTE STUDY

Rajas R. Jadhav*, Vitthal J. Chaware and Vivekkumar K. Redasani

Department of Pharmacology, YSPM's Yashoda Technical Campus Wadhe, Satara, India.

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***Corresponding Author**
Rajas R. Jadhav
Department of Pharmacology, YSPM's Yashoda Technical Campus Wadhe, Satara, India.

ABSTRACT

Aim: The aim of the study to evaluate anti-depressant like effect of Vitamin E and its combination with amitriptyline against reserpine-induced depression in rats. **Materials and Methods:** Reserpine was injected to induce depression in all rats except normal control group. All animals were divided randomly into six groups (6 rats each); Group-I received Vehicle; Group-II received Reserpine; Group-III received Amitriptyline; Group-IV received Vitamin E; Group-V and VI received combination of Amitriptyline and vitamin E. Forced swim test and Actophotometer were used to assess anti-depressant activity. **Result:** In locomotor activity testing, locomotor activity count was found to be increased, when animals were treated with a combination of amitriptyline and vitamin E i.e amitriptyline (5mg/kg) with vitamin E (50mg/kg) and amitriptyline (10mg/kg) with vitamin E (50mg/kg). In forced swim test, latency to immobility time and duration of immobility time was found to be increased and decreased respectively, when animals were treated with a combination of Amitriptyline (5mg/kg) with vitamin E (50mg/kg) as well as amitriptyline (10mg/kg) with vitamin E (50mg/kg). **Conclusion:** Vitamin E showed potentiation and synergistic anti-depressant effect with amitriptyline.

KEYWORDS: Depression, Antidepressant activity, Forced swim test, Reserpine, Vitamin E, Locomotor Activity.

1. INTRODUCTION

Depression is the most prevalent affective disease (defined as disruptions of mood rather than

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thought or cognition); it can range from moderate depression to severe (psychotic) depression with hallucinations and delusions. It is a widespread and exhausting mental disorder that is characterized by low sensitivity, mood, sleeplessness and lack of appetite in enjoyable activities.[1] The Diagnostic and Statistical Manual of Mental Disorders describes the affective symptoms of depression as a loss of interest, which is used to estimate the lifetime incidence of major depression in people.[2]

A rauwolfia indole alkaloid that acts as a sympatholytic and sedative agent but research and clinical trials have shown that reserpine has depressive properties.[3][4] The mechanism of action of reserpine is depletion of monoamines neurotransmitters from nerve endings which may result in poor neuronal excitation and communication leading to depression.[5][6] Therefore, reserpine was used to induce depression in experimental animals.

Antidepressants are the drugs which can elevate mood in depressive illness. All these drugs affect monoaminergic transmission in the brain in one way or the other and have associated properties.[7]

Amitriptyline, a tricyclic antidepressant, is a structurally heterocyclic substance that works as a serotonin-norepinephrine reuptake inhibitor, raising the concentration of these neurotransmitters in the synapse and so successfully treating depression. It is widely accepted that monoamines reuptake inhibition is crucial for its action. By blocking the serotonin and noradrenaline transporters, amitriptyline increases the neurotransmitters in the synapse and hence enhances neurotransmission.[8] Because numerous adverse effects from continuous administration limit therapeutic therapy, it is vital to introduce new targeted medications with claims of improved tolerability and efficacy.[9]

Studies of antioxidant pathways have also suggested that when stress induces biochemical changes, antioxidants can neutralize free radicals and suppress the oxidative stress pathway, eliminating reactive oxygen and nitrogen species (ROS and RNS) that can injure brain neurons. As a result, anxiety and depression symptoms may be reduced as a result of this procedure.[10]

Vitamin E may have an antidepressant impact, according to other research. Vitamin E is a non-enzymatic antioxidant that works in tandem with enzymatic antioxidants like glutathione peroxidase and superoxide dismutase to reduce oxidative stress-related alterations.

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Antioxidant levels in the blood, such as vitamin E, have been linked to sadness and anxiety. Nuts and vegetable oils are common natural sources of vitamin E.[11] Antioxidant supplement treatment has been demonstrated to be useful in individuals with anxiety and depression by increasing antioxidant defense in the biological system.[12][13] In addition, Vitamin E supplementation has few associated adverse events.[14]

The goal of this study was to see how vitamin E combined with amitriptyline delivered immediately affected animal behavior in the forced swim test (FST) and locomotor activity testing in rats. Vitamin E was thought to enhance the antidepressant properties of amitriptyline.

2. MATERIALS AND METHODS

2.1. Animals: This study employed adult male albino rats weighing 180-200 g. They were kept in groups of six at a constant temperature (25°C) and humidity (50%) with a 12-hour light/dark cycle (lights turned on at 7:00 a.m.) and free access to pellets and water. Each animal was utilized only once in each experiment. The institutional ethics committee authorized the experimental protocol.

2.2. Drugs and Treatment: Amitriptyline was procured from (Intas pharma) and vitamin E from MERCK. Reserpine was procured from Sigma-Aldrich. All of the medications were dissolved in RO water and tween 80 and administered one hour before the test. All test solutions were prepared fresh and given orally for 14 days in a volume of 10 mL/kg body weight. The animals were split into six groups (n = 6) at random. The dosages were chosen based on previous research findings.

Group I: received vehicle,

Group II: received reserpine 6 mg/kg, i.p

Group III: received amitriptyline 10mg/kg, p.o

Group IV: received vitamin E 50 mg/kg, p.o,

Group V: received vitamin E 50 mg/kg, p.o + amitriptyline 5 mg/kg, p.o

Group VI: received vitamin E 50mg/kg, p.o + amitriptyline 10mg/kg, p.o

1.1 Locomotor Activity Testing. The locomotor activity was determined. Photoelectric cells connected in a circuit with a counter powered the actophotometer. A count was taken when the animal blocked off the beam of light falling on the photocell. These cut-offs were

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determined during a 10-minute period and the result was utilized as a measure of the animal's locomotor activity.[15]

1.2 Forced Swim Test (FST). The rats were put individually into container of water (usually cylindrical) filled with water according to Porsolt et al FST's procedure. After each test, the water was changed repeatedly to remove fur, urine, and faeces. When the rat stayed afloat in the water without straining and only made little limb movements they were thought to be immobile because they needed to keep their heads above the water's surface. During the 5-minute test, the total length of immobility was measured. The immobility period was estimated by subtracting total time (5 minutes) from time spent fleeing through activities like swimming and climbing. Climbing was defined as upward-directed forepaw motions by the side of the container, whereas swimming was described as movements throughout the container. Treatment with antidepressants shortened the time the animals were motionless and enhanced their escaping behavior.[16][17]

2.3 Statistical Analysis.

All results were presented as mean ± SEM and $p < 0.05$ were considered significant. Data were analysed by one-way ANOVA, followed by Dunnett's post hoc test using Graph Pad Prism version 9.3.1.

3. RESULTS

Locomotor Activity

The effect of Vitamin E and its combination with amitriptyline on locomotor activity was Observed in Table 1.

On day 1

Rats treated with reserpine (6 mg/ kg, i.p) showed a significant decreased in locomotor Activity count as compared to the normal control group. Group III and IV were treated with amitriptyline and Vitamin E respectively, showed a significant increase in locomotor activity, when compared to toxicant group (Group II). Combination of amitriptyline and vitamin E (10mg/kg, p.o & 50mg/kg, p.o) showed a significant increase in locomotor activity as compared to other groups. The results are tabulated in table 1.

Day 1st

Table 1: Effects of Vitamin E and its combination with amitriptyline on locomotor activity.

Gr. No.	Treatment (dose)	No. of locomotor activity
I	Normal Control	340 ±17.12
II	Reserpine (6mg/kg)	142±9.12
III	Amitriptyline (10mg/kg)	180±12.33
IV	Vitamin E (50mg/kg)	154±5.26
V	Amitriptyline + Vitamin E (5mg/kg+50mg/kg)	230±14.33
VI	Amitriptyline + Vitamin E (10mg/kg+50mg/kg)	243±11.33

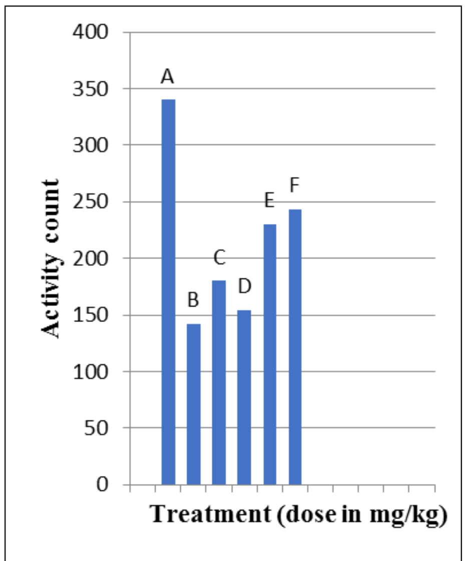

Figure 1: Effects of Vitamin E and its combination with amitriptyline on locomotor activity on 1st day. Values represent the mean ± SEM (*n*=6). Data were analyzed with one-way ANOVA followed by Dunnett's post hoc test.

values are expressed in mean ± SEM, where n=6

p<0.05, compared with normal control group

Day 7th

On day 7th, the locomotor activity count was found to be significantly decreased, when animals were treated with reserpine (6 mg/kg, i.p) as compared to normal control group. The effect of Amitriptyline and vitamin E alone showed significantly increase in locomotor activity count as compared to toxicant group (Group II). Better result were observed when Amitriptyline combined with vitamin E at doses of (10 mg/kg, p.o& 50 mg/kg, p.o) respectively. The results are tabulated in table 2.

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Table 2: Effects of Vitamin E and its combination with amitriptyline on locomotor activity.

Gr. No.	Treatment (dose)	No. of locomotor activity
I	Control	327±12.32
II	Reserpine (6mg/kg, i.p)	90±7.78
III	Amitriptyline (10mg/kg, p.o)	205±10.43
IV	Vitamin E (50mg/kg, p.o)	163±9.14
V	Amitriptyline + Vitamin E (5mg/kg, p.o+50mg/kg, p.o)	269±13.5
VI	Amitriptyline + Vitamin E (10mg/kg, p.o+50mg/kg, p.o)	274±7.6

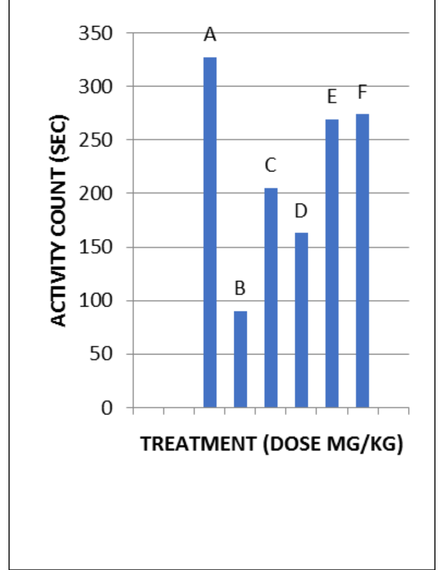

Figure 2: Effects of Vitamin E and its combination with amitriptyline on locomotor activity on 7th day. Values represent the mean ± SEM (*n*=6). Data were analyzed with one-way ANOVA followed by Dunnett's post hoc test.

Values are expressed in mean ± SEM, where *n*=6.

$p<0.05$, compared with normal control group

On day 14

At the end of treatment phase, the group II animals injected by Reserpine showed significant decrease in locomotor activity as compared to normal control animals. The behavioural effect on day 14 was shown in table 3. Amitriptyline and vitamin E alone showed a significantly increase in locomotor activity count as compared to toxicant group (Group II). Co-administration of Amitriptyline and Vitamin E (10 mg/kg, p.o & 50mg/kg, p.o) showed a significant increased effect as compared to toxicant group (Group II).

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Table 3: Effects of Vitamin E and its combination with amitriptyline on locomotor activity.

Gr. No.	Treatment (dose)	No. of locomotor activity
I	Control	289±14.33
II	Reserpine (6mg/kg)	165±9.88
III	Amitriptyline (10mg/kg)	235±18.12
IV	Vitamin E (50mg/kg)	239±14.12
V	Amitriptyline + Vitamin E (5mg/kg+50mg/kg)	357±19.88
VI	Amitriptyline + Vitamin E (10mg/kg+50mg/kg)	364±24.55

Day 14th graphical representation of (actophotometer)

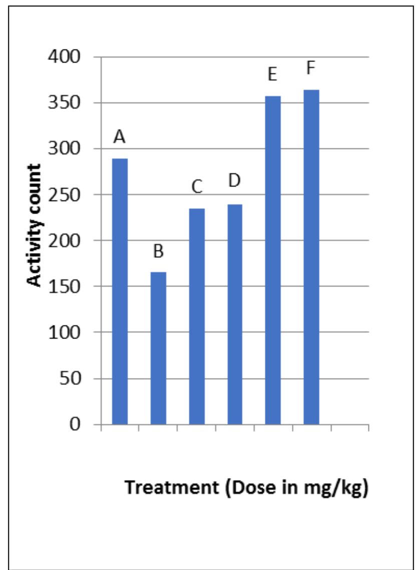

Figure 3: Effects of Vitamin E and its combination with amitriptyline on locomotor activity on 14th day. Values represent the mean ± SEM (*n*=6). Data were analyzed with one-way ANOVA followed by Dunnett's post hoc test.

Values are expressed in mean ± SEM, where *n*=6.

$p<0.05$, compared with normal control group.

2. Forced swim test

Day-1st

Latency to immobility time and duration of immobility time was found to be significantly decreased and increased respectively as compared to normal control group, when animals treated with Reserpine (6mg/kg, i.p). Group III and IV animals were treated with Amitriptyline and Vitamin E showed significant increased latency to immobility time and decreased duration of immobility time. Combination of Amitrptyline with Vitamin E (10

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mg/kg, p.o & 50 mg/kg, p.o) showed significant increased and decreased latency to immobility time and duration of immobility time respectively. The results are tabulated in table 4.

Table 4: Effects of Vitamin E and its.
combination with amitriptyline using forced swim test.

Gr. No.	Treatment (Dose)	Latency to immobility (sec)	Duration of Immobility time (sec)
I	Control	35±2.33	67±5.33
II	Reserpine (6mg/kg)	22±1.25	102±9.33
III	Amitriptyline (10mg/kg)	37±2.45	85±7.58
IV	Vitamin E (50mg/kg)	41±4.12	93±8.85
V	Amitriptyline + Vitamin E (5mg/kg+50mg/kg)	30±1.23	82±5.26
VI	Amitriptyline + Vitamin E (10mg/kg+50mg/kg)	39±2.56	77±7.33

Day 1st Graphical Representation (FST)

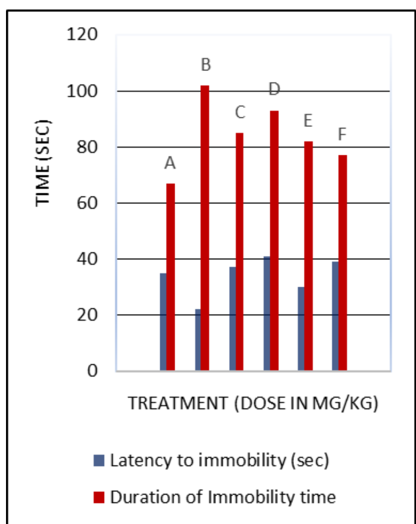

Figure 4: Effects of Vitamin E and its combination with amitriptyline using FST on1st day. Values represent the mean ± SEM (*n*=6). Data were analyzed with one-way ANOVA followed by Dunnett's post hoc test.

Values are expressed in mean ± SEM, where *n*=6.

p<0.05, compared with normal control group.

Day-7th

On day 7th, Latency to immobility time and duration of immobility time was found to be significantly decreased and increased respectively as compared to normal control group, when animals treated with Reserpine (6mg/kg, i.p). Group III and IV animals were treated

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with Amitriptyline and Vitamin E showed significant increased latency to immobility time and decreased duration of immobility time. Better result were observed when Amitriptyline combined with vitamin E at doses of (10 mg/kg, p.o & 50 mg/kg, p.o) respectively. The results are tabulated in table 5.

Table 5: Effects of Vitamin E and its combination with amitriptyline using forced swim test activity.

Gr. No.	Treatment (Dose)	Latency to immobility (sec)	Duration of Immobility time (sec)
I	Control	27±3.17	56±6.11
II	Reserpine (6mg/kg)	16±4.31	153±9.34
III	Amitriptyline (10mg/kg)	38±2.12	137±7.56
IV	Vitamin E (50mg/kg)	32±5.6	145±8.16
V	Amitriptyline + Vitamin E (5mg/kg+50mg/kg)	45±7.13	127±5.43
VI	Amitriptyline + Vitamin E (10mg/kg+50mg/kg)	56±3.14	120±7.13

Day 7th Graphical representation (FST)

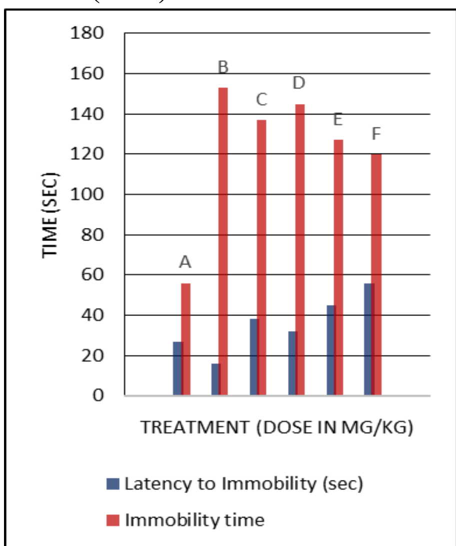

Figure 5: Effects of Vitamin E and its combination with amitriptyline using FST on 7th day. Values represent the mean ± SEM (*n*=6). Data were analyzed with one-way ANOVA followed by Dunnett's post hoc test.

Values are expressed in mean ± SEM, where *n*=6.

$p<0.05$, compared with normal control group.

Day-14th

At the end, latency to immobility time and duration of immobility time was found to be significantly decreased and increased respectively, when animals were injected by Reserpine

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(6mg/kg, i.p) as compared to normal control group. The effect of amitriptyline and vitamin E alone showed a significant increased latency to immobility time and decreased duration of immobility time as compared to toxicant group (Group II). The co-administration of amitriptyline and vitamin E (10 mg/kg, p.o and 50mg/kg, p.o) showed a prominent effect as compared to toxicant group (Group II).The results was found to be dose dependent and are tabulated in table 6.

Table 6: Effects of Vitamin E and its combination with amitriptyline using forced swim test.

Gr. No.	Treatment (Dose)	Latency to immobility (sec)	Latency to Immobility time (sec)
I	Control	42±3.58	71±6.66
II	Reserpine (6mg/kg)	32±3.33	147±12.45
III	Amitriptyline (10mg/kg)	45±5.12	121±11.23
VI	Vitamin E (50mg/kg)	39±3.33	127±14.15
V	Amitriptyline + Vitamin E (5mg/kg+50mg/kg)	54±4.58	87±7.88
VI	Amitriptyline + Vitamin E (10mg/kg+50mg/kg)	59±3.66	83±4.55

Day 14th Graphical representation (FST)

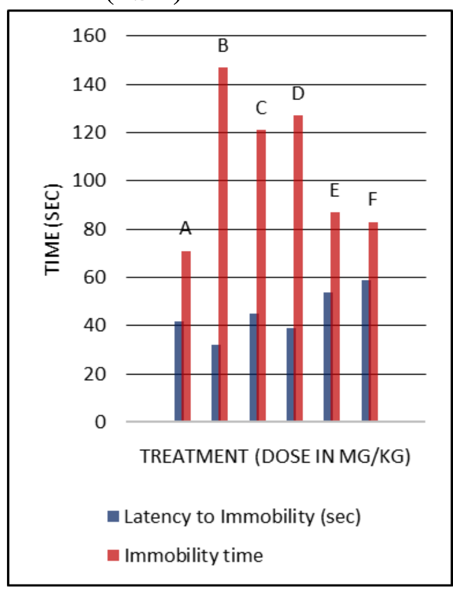

Figure 6: Effects Vitamin E and its combination with amitriptyline on FST on 14th day. Values represent the mean ± SEM (*n*=6). Data were analyzed with one-way ANOVA followed by Dunnett's post hoc test.

Values are expressed in mean ± SEM, where *n*=6.

$p<0.05$, compared with normal control group.

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4. DISCUSSION

The goal of this study was to see if vitamin E had antidepressant properties and to see how different dosages and combinations of amitriptyline medications affected reserpine-induced depression. The intraperitoneal administration of reserpine 6 mg/kg, i.p was proven to be a progressive model of depression and was employed to produce depression in our investigation.

Amitriptyline, a tricyclic antidepressant, is used as a standard medicine because it increases locomotor activity count in the actophotometer as well as increases latency to immobility time and increases duration of immobility time in the forced swim test, some studies have also demonstrated that amitriptyline reduces the immobility time in rodents on the first day of behavioural test.[18][19] Monoamine reuptake inhibition is thought to be essential for its effect. Amitriptyline increases neurotransmitters in the synapse and hence improves neurotransmission by inhibiting serotonin and noradrenaline transporters.[20]

Considering the role of oxidative and nitro-sative stress in the pathophysiology of depression and vitamin E's possible antioxidant properties, we tested vitamin E's antidepressant-like impact in an animal model of depressive-like behaviour generated by reserpine. Free radicals are extremely reactive chemical entities that are produced by inflammation and mitochondrial oxidative reactions. Reactive Oxygen Species (ROS) may react with macromolecules of the cell such as fatty acids, DNA, protein, and other macromolecules, causing damage to these macromolecules, when these radicals become excessive or when the antioxidant system is depleted.[21] ROS may have a role in the pathophysiology of depression through a variety of processes, including tissue damage, inflammation, neuro-degeneration, immunological responses triggered by tissue damage, and apoptosis.[22]

Clinical and preclinical research in cell and animal models provide support for oxidative stress in major depressive illness. These studies either look at oxidative homeostasis in patients with depressive disorder indirectly through an increase in neuronal damage caused by increased free radicals, such as lipid peroxidation and DNA strand-brakes.

All of the groups treated with reserpine 6 mg/kg experienced depression. On the first day of administration, all reserpine-treated groups showed a minor increase in immobility duration in FST and Actophotometer, but a considerable increase on the seventh day. In comparison to the toxicant group (Group II), those treated with amitriptyline, vitamin E, or a combination of

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both showed meaningful impact. On fourteenth day it increases and decreases latency to immobility time, duration of immobility time respectively and also increases locomotor activity much more compared on first day of administration.

To our knowledge, this is the first study to show a potentiation and synergistic impact of vitamin E as an antidepressant medicine. Patients resistant to traditional therapies can be treated with vitamin E (50 mg/kg) and amitriptyline (10 mg/kg). Additionally, vitamin E (50 mg/kg) in conjunction with a sub-effective dosage of amitriptyline (5 mg/kg) may reduce antidepressant doses and hence adverse effects. This is critical in situations of infantile depression or postpartum depression, where drug safety is a concern.

5. CONCLUSION

In conclusion, the current investigation confirms that vitamin E potentiate anti-depressant effect of Amitriptyline in the forced swim test and Actophotometer. These findings might be useful in the development of novel therapeutic options as well as in clinical practice. The synergistic impact may result in a better response for those with treatment-resistant depression, as well as a reduction in the severity of adverse effects from low-dose antidepressants.

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Lipid Lowering Effect of Alpha Adrenoreceptor Blocker and Antidiabetic Drug in Experimental Animals

Swapnali S. Jagtap[1]*, Vitthal J. Chaware[2], Vivekumar K. Redasani[3]

[1]Department of Pharmacology, YSPM's Yashoda Technical Campus Wadhe, Satara, India
[2]Head of Pharmacology, YSPM'S Yashoda Techninal Campus, Wadhe, Satara, India
[3]Principle, YSPM'S Yashoda Technical Campus, Wadhe, Satara, India

Email ID: jagtapswapnali24@gmail.com

Abstract: Lipid Lowering Effect of Alpha Adrenoreceptor Blocker and Antidiabetic Drug in Experimental Animals. **Methods:** Hyperlipidemia was induced by intraperitoneal injection of poloxamer 407 at a dose of 400mg/kg body weight in wistar albino rats. Drugs treatment were done by oral gavage for 3 days. At the end of the study, animals were kept fasted overnight and then blood sample was collected. The serum cholesterol (TC), triglycerides (TC), HDL, LDL, VLDL were calculated. **Results:** From the present investigation, it was observed that pioglitazone and terazosin drug have shown significant reduction in serum cholesterol, triglycerides, LDL, VLDL and increase in HDL level in p-407 induced hyperlipidemia.**Conclusion:** It is concluded that Pioglitazone and terazosin may possess antihyperlipidemic activity in Poloxamer 407 induced Hyperlipidemic Rats.
Keywords : Hyperlipidemia, Poloxamer 407, pioglitazone, Terazosin, Atorvastatin, lipid profile.

INTRODUCTION

Hyperlipidemia, also known as hyperlipoproteinemia, is characterised by unusually high amounts of lipids and lipoproteins in the blood.[1] Any aberrant lipid levels are included in this type of dyslipidaemia, which is the most frequent. Hyperlipidemia is basically divided into two types viz. primary and secondary type. [2,3] Primary hyperlipidemia is caused by genetic factors (for example, a mutation in a receptor protein), whereas secondary hyperlipidemia is caused by extrinsic factors such as diabetes. Because of their influence on atherosclerosis,

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Yashoda Technical Campus
Satara

lipid and lipoprotein abnormalities are frequent in the general population and are regarded as an unmodifiable risk factor for cardiovascular disease. Furthermore, some types may put you at risk for acute pancreatitis.[1]

According to the world health organisation (WHO), excessive blood cholesterol is responsible for around 56 percent of all occurrence of cardiovascular disease (CVD) worldwide, resulting in nearly 4.4 million death per year. When compared to 1990, it is estimated that more than 62.4 percent of person in India died from cardiovascular disease. [1,4]

Hyperlipidemia is a secondary metabolic disorder linked to diabetes that also increases the chance of developing the disease. Aside from the cause-effect link with diabetes, high levels of triglycerides, cholesterol, and low density lipoprotein in the blood are risk factors for cardiovascular disorders such as atherosclerosis, hypertension, and coronary heart disease.[1] Hyperlipidemia related to increased oxidative stress causing significant production of oxygen free radicals, which may lead to oxidative modification in LDL ,which present a significant function in the initiation and progression of atherosclerosis and associated cardiovascular diseases.[5,6]

In addition, hyperlipidemia is induced by secondary effect of diabetes therefore by secondary effect of diabetes therefore, the agent having some antidiabetic effect also showed favourable effect to hyperlipidemia. [7,8] Type 2 diabetes is treated with thiazolidinediones (TZD), pioglitazone, and rosiglitazone. Pioglitazone is a less strong agonist of the peroxisome proliferator-activated receptor gamma (PPAR-) than rosiglitazone, but it is also beneficial in lowering fasting blood glucose and HbA1c levels. Pioglitazone, a commonly used antidiabetic, has been proven to improve HDL and decrease LDL and TG in diabetics, in addition to improving glycemic control. All anti-diabetic medicines affect lipid profiles differently, however pioglitazone has a better lipid-lowering effect than other anti-diabetic treatments, including rosiglitazone. In type 2 diabetic individuals, pioglitazone has been proven to have better cardiovascular advantages than rosiglitazone and glimipride. When compared to atorvastatin alone, co-administration of pioglitazone with atorvastatin improved the lipid profile in non-diabetic patients with high cardiovascular risk. Pioglitazone has a minor PPAR alpha (PPAR-) agonist effect in addition to PPAR-, which may be responsible for improved lipid and cardiovascular profiles.[9]

Alpha-blocking drugs are used as first-line treatment for benign prostatic hyperplasia (BPH), one of the most common causes of consultation for obstructive or irritative urological problems in middle-aged and elderly men. They are also used as second-line treatment for uncontrolled arterial hypertension, in monotherapy or in combination.[10]

In vitro investigations have shown that terazosin metabolites have antioxidant characteristics, which could be effective in preventing atherosclerosis in hypertensive patients, especially when other comorbidities like dyslipidaemia and diabetes are present. Then there's evidence of doxazosin's hypocholesterolaemia and antioxidant properties, which haven't been established with other alpha-blockers but suggest a possible benefit against endothelial dysfunction in a variety of situations.[10]

Poloxamer 407(P-407) is a non-ionic surfactant made up of polyoxyethylene and polyoxypropylene units in a block copolymer. It's known for its biocompatibility and capacity to administer medications for a variety of diseases, and it works as a barrier against post-surgical adhesion. P-407 possesses remarkable thermo-reversible characteristics, in that it is liquid at room temperature but aggregates and forms a gel at body temperature before producing micelles. This temperature-dependent micelle and gel formation ability makes them commercially beneficially in personal care products including mouthwashes, deodorants and skin care products, as well as an inactive substance that can be used as a vehicle or media for a range of medicinal preparations [11]

In the present investigation we determined the lipid lowering effect of the alpha-blocking drugs terazosin and antidiabetic drug i.e. pioglitazone in hyperlipidemia model.

MATERIALS AND METHODS:

Ethics approval: The study was approved by institutional ethics committee of YSPM's YTC, Satara MH India.

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Drug and Chemical: Terazosin tablets (Intas Pharmaceutical Ltd), Pioglitazone tablets (Ontop Pharmaceutical PVT.Ltd), Atorvastatin tablets (Emcure Pharmaceutical Ltd), Poloxamer 407 (Ozone Pharmaceutical Ltd) were used for these research study.

Animals:

Inbred 30 Wistar albino rats (150–220 gm) were selected for present study. The animals were housed at room temperature (22-28 ºC) 12 hr dark and light cycle and given standard laboratory feed and water *ad-libitum*. The study was approved and conducted as per the norms of the Institutional Animal Ethics Committee (25/12/2017/CPCSEA). Animals were maintained as per committee for the purpose of control and supervision of experiments on animals guidelines. [12,13]

Induction of hyperlipidemia:

The inducing agent was poloxamer 407. Before administration, P-407 was completely dissolved in water and refrigerated overnight to aid its complete dissolution. The syringe and needle to be used for the induction was cooled to avoid gelation within the syringe during injection.[11]

Experimental procedures:

Poloxamer 407-induced hyperlipidemic model

To render the animals hyperlipidemic, the rats were subjected to a 6 h-fast. Next, the rats were administered an intraperitoneal injection (i.p.) of a 400 mg/kg dose of poloxamer 407. [14,15] It had been prepared by combining the agent with saline or water for injection and then refrigerated over-night to facilitate dissolution of poloxamer 407. Starting two hours after the administration of the poloxamer 407, the rats were treated with prepared samples once daily for 3 days by oral gavage.[15]

Animals grouping and treatment:

A total of 30 rats were used. The rats were randomly divided into 5 groups. Each group contained 6 rats.

Group I: Normal Control rats fed with normal chow and distilled water (NC)

Group II: Hyperlipidemic Control rats induced without treatment (HC)

Group III: Hyperlipidemic rats treated with the standard drug (Atorvastatin 10mg/kg p.o.)

Group IV: Hyperlipidemic rats treated with the antidiabetic drug (pioglitazone 10mg/kg p.o.)

Group V: Hyperlipidemic rats treated with the alpha blocker drug (terazosin 5mg/kg p.o.)

Biochemical estimations:

Blood was collected by retro-orbital sinus puncture, under mild ether anaesthesia in the experimental models. The collected samples were centrifuged for 15 minutes at 2500rpm. Then serum samples were collected and analysed for serum Total Cholesterol (TC), Triglycerides (TG), High Density Lipoprotein Cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C) and Very Low-Density Lipoprotein Cholesterol (VLDL-C).[12]

Statistical analysis:

The data was statistically analysed using one-way ANOVA followed by Tukey's multiple test. The results were expressed as Mean ± SEM (n=6). A value $P < 0.05$ was considered to be significance. [16]

RESULTS AND DISCUSSION

Table 1: Effect of Pioglitazone and Terazosin on lipid profile of P-407 induce hyperlipidemia

Group	Cholesterol (mg/kg)	Triglycerides (mg/kg)	HDL (mg/kg)	LDL (mg/kg)	VLDL (mg/kg)
Normal control	142 ±13.78	99 ±5.80	44 ±3.12	86 ±7.66	26 ±1.66
Toxicant control (Poloxamer 407)	322 ±22.11	198 ±12.33	24.00 ±1.44	185 ±12.86	56 ±4.33
Standard (Atorvastatin)	158 ±14.33	122 ±14.18	41 ±3.22	91 ±8.63	27 ±1.18
Test I(Pioglitazone)	202 ±18.22	143 ±12.44	33 ±2.11	134 ±11.12	41 ±3.33
Test II (Terazosin)	283 ±17.33	167 ±15.33	29 ±2.33	165 ±14.33	51 ±4.12

The values are expressed as a mean ± SEM, n=6, $p<0.05$ when compare to normal control and hyperlipidemic control.

Graphical Representation:

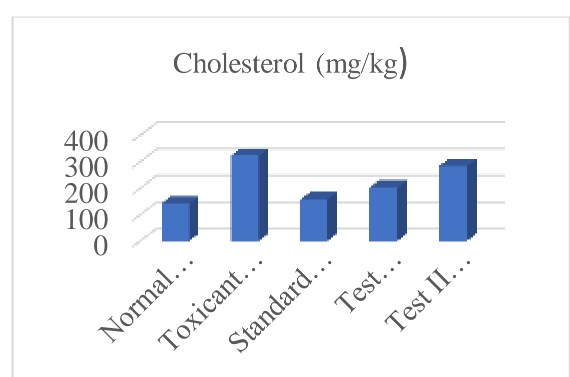


Fig.No.1 Effect of Pioglitazone and Terazosin on Total Cholesterol

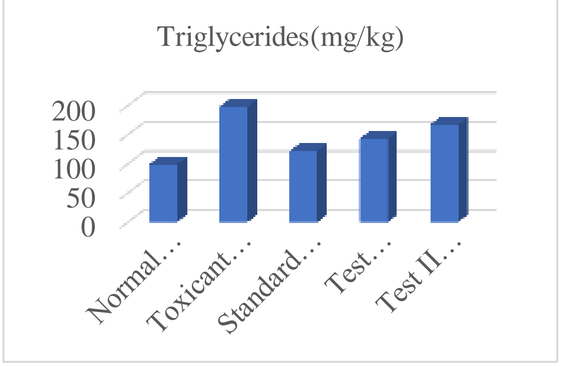


Fig.No.2 Effect of Pioglitazone and Terazosin on Triglycerides

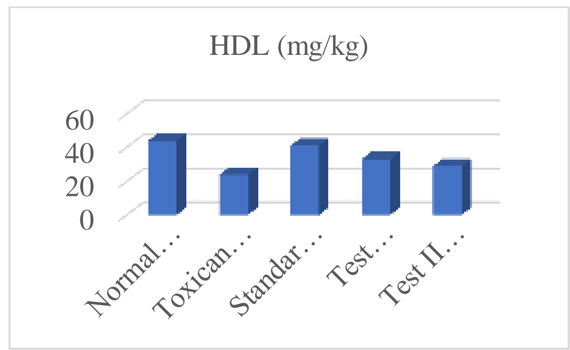


Fig.No.3 Effect of Pioglitazone and Terazosin on HDL

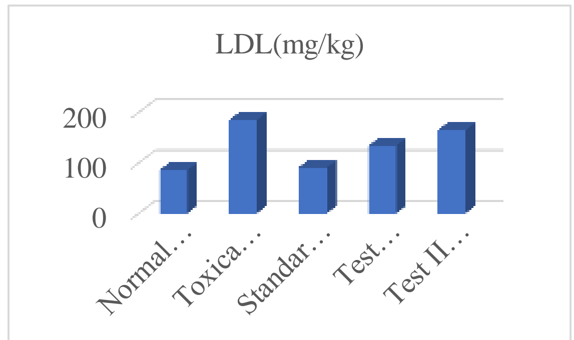
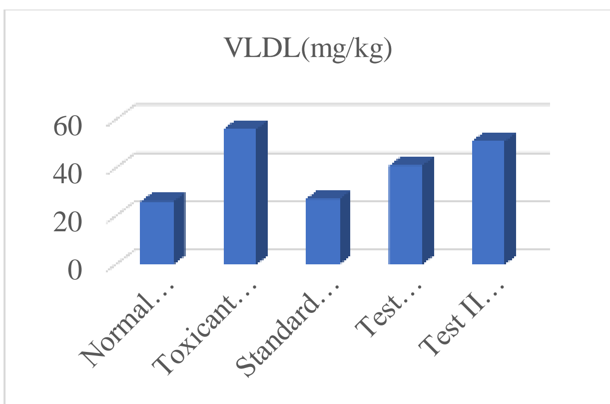


Fig.No.4 Effect of Pioglitazone and Terazosin on LDL



Lipids are water-insoluble organic molecules that are soluble in organic solvents. Lipids provide a variety of tasks, including chemical messengers, energy storage and provision, temperature regulation, and membrane lipid layer development. Hyperlipidemia is defined as an unusually high level of lipids, such as total cholesterol (TC), triglycerides (TG), and lipoproteins (lipoproteins) [17]. Hyperlipidemia-related diseases are substantial risk factors for the development of cardiovascular disease (CVD) [18].

Hyperlipidemia is a risk factor for atherosclerosis beginning and progression [19] as well as a high-risk factor for coronary heart disease development. As a result, the causal hyperlipidemia can be targeted for prevention or therapy of such illness. The abnormal high concentration of serum lipid is mainly due to increase in the mobilization of free fatty acids from the peripheral depots. [20,21]

Poloxamer 407 is non-ionic surfactant and is nontoxic to cellular membrane, was used successfully to induce hyperlipidemia in previous studies it causes effects by activating HMG CoA enzyme A. Poloxamer 407 a block copolymer composed of a hydrophobe that is flanked on each side with hydrophillic polyoxyethylene units. Our previous findings demonstrated that elevation in plasma TG was more sensitive than elevation in total plasma cholesterol following P-407 administration.[22]

Pioglitazone is a glucose-lowering medication that works as an agonist of peroxisome proliferator–activated receptor gamma.[13] All anti-diabetic drugs have varying effect on lipid profile but overall pioglitazone has shown more favourable lipid-lowering effect in comparison to other antidiabetics.[23]

Terazosin, which is structurally similar to prazosin, is a novel selective alpha 1- adrenoceptor antagonist.[24] The association between thyroid hormone imbalance and blood lipids encompasses processes such as beta oxidation at the muscle and liver level, as well as increasing the turnover of LDL, which could explain the decrease in cholesterol levels. Terazosin would cause a change in thyroid hormone levels, allowing us to notice a reduction in cholesterol and triglyceride levels.[10]

Group I administered with saline or water considered as normal control group. Group II administered with poloxamer 407 showed significant increase in lipid profile level except HDL level as compared to normal control group. Group III administered with atorvastatin showed significant decrease lipid profile except HDL level which is good cholesterol as compared toxicant control group. Group IV treated with pioglitazone significantly decreased TC, TG, LDL, VLDL and increased HDL level as compared to hyperlipidemic control group. Group V treated with terazosin significantly decreased TC, TG, VLDL, LDL and increased HDL level as compared to toxicant control group.

Conclusion:

In conclusion the present study has demonstrated that pioglitazone and terazosin are found to be of potential anti-hyperlipidemic activity in poloxamer-407 induced hyperlipidemia in wistar rats and it is observed that significant reduction of cholesterol, triglycerides, LDL, VLDL and increases HDL cholesterol level. According

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Satara

to above study we can conclude that pioglitazone showed more significant effect as compared to terazosin. So pioglitazone is beneficial in preventing atherosclerotic cardiovascular diseases.

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HEPATOPROTECTIVE EFFECT OF LYCOPENE AGAINST PARACETAMOL-INDUCED HEPATIC DAMAGE IN ALBINO RATS

Shravani P. Pawar*, Vitthal J. Chaware and Vivekkumar K. Redasani

Department of Pharmacology, YSPM's Yashoda Technical Campus Faculty of pharmacy, Wadhe, Satara, India.

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***Corresponding Author**

Shravani P. Pawar

Department of Pharmacology, YSPM's Yashoda Technical Campus Faculty of pharmacy, Wadhe, Satara, India.

ABSTRACT

Aim: The hepatoprotective effect of Lycopene was evaluated against paracetamol induced hepatic damage in albino rats. **Materials and Methods:** Liver function tests and biochemical parameters were estimated usingstandard kits. Livers were quickly removed and fixed in 10% formalin and subjected to histopathological studies. **Results:** There was a significant ($p < 0.05$) reduction in serum bilirubin levels with silymarin and lycopene 10mg/kg treated groups signifying protection against hepatic damage, lycopene 5mg/kg treated groups also showed significant change in bilirubin level. Similarly, significant ($p < 0.05$) reduction in the levels of serum transaminases were observed with all the treatment groups though more evident in the positive control and lycopene 10mg/kg treated groups. **Conclusion:** The results of this study strongly indicate that Lycopene may possess hepatoprotective action against paracetamol induced hepatic damage in rats.

KEYWORDS: Paracetamol, Lycopene, Silymarin, Heptoprotective.

INTRODUCTION

The liver is of vital importance in intermediary metabolism and in detoxification and elimination of toxic substances. The liver is often affected by a multitude of environmental pollutants and drugs, all of which place a burden on this vital organ and can damage and weaken it, eventually leading to diseases like hepatitis or cirrhosis.[1]

Paracetamol's hepatotoxicity is caused by its reactive metabolite. N-acetyl-p-benzoquinone imine (NAPQI), which causes oxidative stress and glutathione (GSH) depletion. Paracetamol

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toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P450.[2] Introduction of cytochrome or depletion of hepatic glutathione is a prerequisite for paracetamol-induced hepatotoxicity.[3,4,5] In spite of tremendous strides in modern medicine, the treatment of liver disorders is inadequate and many formulations containing herbal extracts are used for regeneration of hepatic cells and for protection of the liver against damage.[6] Hepatic damage is associated with distortion of its metabolic functions and it is still a major health problem.[7]

Unfortunately many synthetic drugs used in the treatment of liver diseases are inadequate and also cause serious side effects.[8] In view of severe undesirable side effects of synthetic agents, there is growing interest in evaluating traditional herbal medicines that are claimed to possess hepatoprotective activity. A single drug cannot be effective for all types of severe liver diseases. Therefore, an effective formulation using indigenous medicinal plants has to be developed with proper pharmacological experiments and clinical trials.[9]

The main objective of this study is to further understand the mechanism of lycopene's antioxidant action by evaluating the protective effect of orally administered lycopene pre-treatment on paracetamol induced rats. paracetamol is a hepatotoxic agent used to induce liver injury in experimental animals to check the efficiency of potential hepatoprotective agents.[10] The present study investigates the activity of the Lycopene against paracetamol-induced toxicity in comparison with silymarin a well-known antihepatotoxic agent.

MATERIALS AND METHODS

Experimental design

This experimental study was carried in models of paracetamol induced hepatotoxicity in albino rats. Lycopene was evaluated in paracetamol induced hepatic damage. The effects of lycopene was compared with silymarin, a proven hepatoprotective agent in this model of hepatotoxicity.

The study was conducted in strict accordance with the study protocol and CPCSEA guidelines Study animals were housed in the Central Animal House of our Institute, in an air-conditioned area with 12-15 filtered fresh air changes, temperature 22-30°C, relative humidity 30-70% six rats per cage were housed in polypropylene cages having husk paddy as the bedding, during the study. Twelve hourly light and dark cycles were maintained.

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The model was standardized and hepatic damage was confirmed in the model. The effects of lycopene were evaluated in experimental models of paracetamol induced hepatic damage 30 albino rats of either sex weighing between 150-200 grams were used for the entire study. Lycopene was used in two doses of 5mg/kg and 10mg/kg based on the dose animal studies of lycopene in previous as hepatoprotective. Lycopene was administered orally. Daily, suspended in 0.5% CMC. Silymarin was administered in the dose of 50mg/kg, orally.

The effects of lycopene were evaluated in paracetamol induced hepatic damage, using silymarin as positive control. 24 Wistar rats were randomly allocated into four group's namely toxicant control (paracetamol 2gm/kg), silymarin (50mg/kg), lycopene (5mg/kg) and lycopene. (10mg/kg), each group containing 6 rats. The study drugs were administered for a duration of 7 days. On the 8th day, Induction of hepatic damage was carried out with paracetamol given orally in the single dose of 2g/kg. 24 hours following the administration of paracetamol, 2ml of blood was collected by puncturing the retro-orbital sinus and biochemical investigations was performed. Then the rats were euthanized by administering ketamine intraperitoneally. The liver was dissected out, washed in cold saline and blotted dry by placing it on tissue paper Weight and volume of liver was measured and processed further for histopathological examination.

Assessment of liver function parameters

At the end of the experimental period, animals were sacrificed by cervical decapitation under mild ketamine anesthesia, blood was collected and the serum was separated by centrifuging at 300 rpm for 10 min. The collected serum was used for the assay of marker enzymes. The serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were estimated by the method of Reitman and Frankel.[12] Alkaline phosphatase (ALP) was determined by the method of Kind and King.[13]

Assessment of biochemical parameters

The total bilirubin was estimated by Method of Malloy and Evelyn.[16] Immediately after sacrificing the animal, the liver was excised from the animals, washed in ice-cold saline, and the weight of the liver was recorded.

Histological studies

Livers were quickly removed and fixed in 10% formalin, dehydrated in gradual ethanol (50%–100%), cleared in xylene and embedded in paraffin. Sections (4–5 mm thick) were prepared and

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then stained with hematoxylin and eosin dye for photo microscopic observations of the liver histologic architecture of the control and treated rats.

Statistical analysis

The results were expressed as mean ± standard deviation (S.D). Differences in liver function parameters and biochemical parameters were determined by factorial one-way ANOVA. Individual groups were compared using Tukey's test. Differences with $P<0.05$ were considered statistically significant.

RESULTS

There were no macroscopic changes observed in the liver any of the study groups. There was statically significant decrease in the liver weight and volume observed with grips that received silymarin 50mg/kg and lycopene 10mg/kg when compared with toxicant control. (Table 1)

Table 1: Effect of lycopene on liver Weight and Volume in rat model of paracetamol induced hepatotoxicity.

Groups	Liver weight (gm/100gm body weight)	Liver volume (ml/100gm body weight)
Normal control	4.82±0.11	5.62±0.24
Toxical control	5.81±0.12	9.62±0.17
Standard (Silymarin 50mg/kg)	3.81±0.12	5.64±0.17
Test I (Lycopene 5mg/kg)	4.62±0.43	5.13±0.22
Test II (Lycopene (10mg/kg)	3.95±0.41	5.90±0.18

All values represent Mean ±SD (n=6).

Biochemical parameters

There was a significant ($p<0.05$) reduction in serum bilirubin levels with silymarin and both doses of lycopene 5mg /kg and 10mg/kg treated groups signifying protection against hepatic damage. Similarly significant ($p<0.05$) reduction in the levels of serum transaminases were observed with positive control and lycopene 5 and 10mg /kg treated groups. (Table 2)

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Table 2: Effect of lycopene on biochemical parameters in rat model of paracetamol induced hepatotoxicity.

Groups	Serum bilirubin (mg/dl)	Aspartate transaminase (IU/ml)	Alanine transaminase (IU/ml)	Alkaline phosphatase(IU/ml)
Normal control	0.53±0.03	81.10±8.52	75.43±8.33	90.96±6.66
Toxicant control	0.93±0.08	308.40±17.10	149.05±11.43	248.01±22.23
Standard (Silymarin 50mg/kg)	0.28±0.04	140.02±12.35	62.53±7.10	126.02±10.40
Test I (Lycopene 5mg/kg)	0.48±0.03	198.26±12.16	68.32±05.28	180.03±15.33
Test II (Lycopene 10mg/kg)	0.36±0.06	137.24±13.39	66.42±06.32	122.01±11.30

All values represent Mean ±SD (n=6) $p<0.05$ using one way ANOVA with post hoc Tukey's test (versus toxicalnt control)

Table 3: Histopathological changes.

Groups	No of animals showing						
	Degeneration				Necrosis		
	0	I	II	III	0	I	II
Normal control	0	4	2	0	1	5	0
Standard (Silymarin 50mg/kg)	1	5	0	0	4	2	0
Test I (Lycopene 5mg/kg)	0	4	2	0	1	5	0
Test II (Lycopene 10mg/kg)	1	4	1	0	4	2	0

Out of the six animals in the normal group four showed grade I degeneration while the remaining, two showed grade II degeneration. Five animals showed presence of 1-2 necrotic cells per high power field (Grade 1) while one animal showed no necrosis. Similar changes were seen in the group that received lycopene in the dose of 5mg/kg with regard to the number of animals.

In the silymarin treated group, 4 animals showed no necrosis while two animal showed necrosis and Minimal degenerative changes were seen in 5 animals.

In lycopene 10mg/kg treated group, one animal showed no degeneration and grade II degeneration each, with remaining 4 animals showing grade I degeneration. Two animal in the lycopene 10mg/kg treated group showed necrosis while 4 showed near normal hepatic

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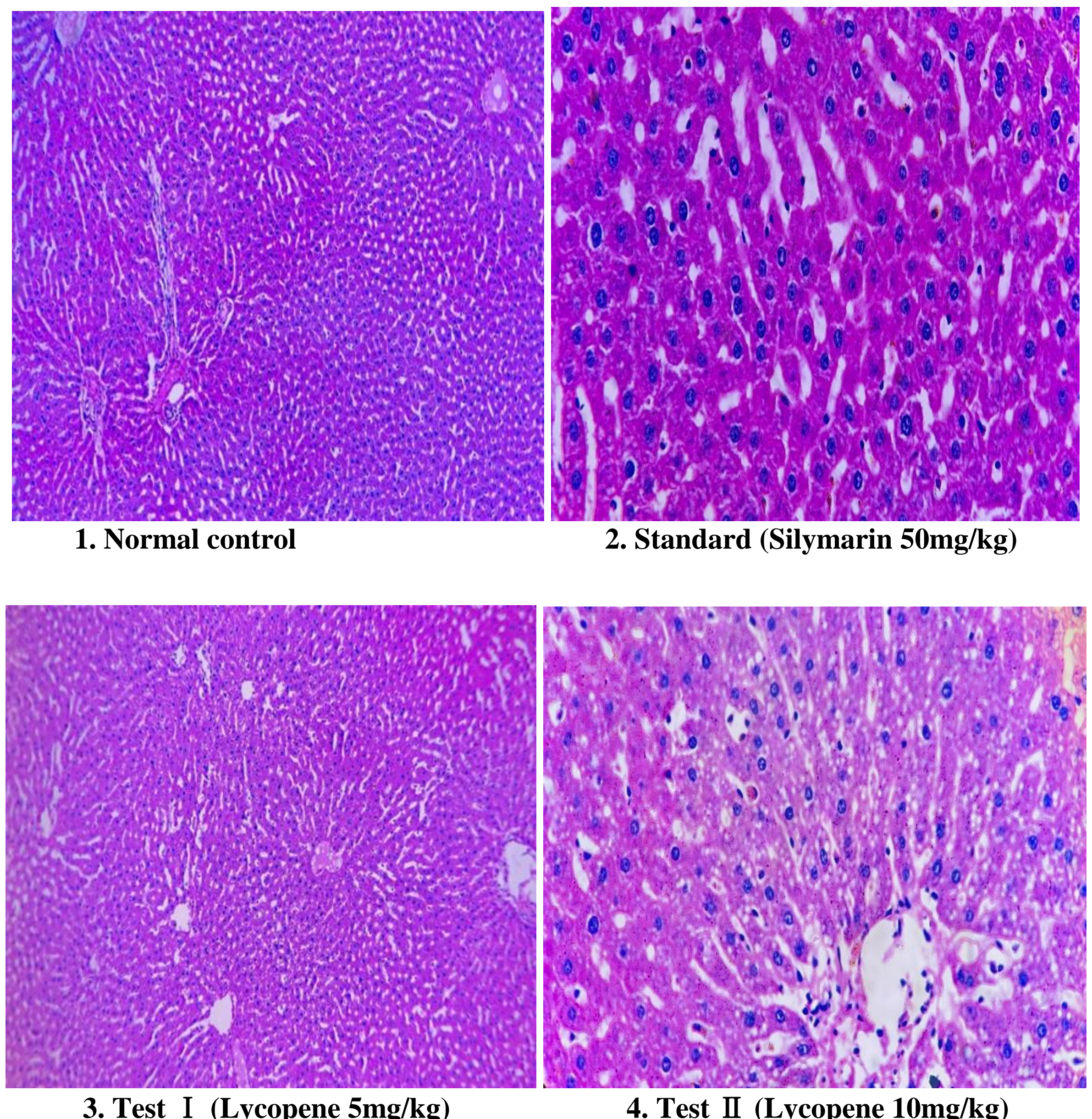
parenchyma. (Table 3, Figure 1).



1. Normal control 2. Standard (Silymarin 50mg/kg)

3. Test Ⅰ (Lycopene 5mg/kg) 4. Test Ⅱ (Lycopene 10mg/kg)

Figure 1: (1 to 4) effects of study drugs on histopathology in rat model of paracetamol induced hepatic damage.

DISCUSSION

While standardizing this model, Serum bilirubin, AST, ALT and ALP as biochemical parameters were chosen. Morphological parameters such as liver weight and liver volume were measured to find changes in liver morphology. Structural alterations the liver due to ongoing insults was confirmed by doing histopathological examination of liver at the end of study.

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During standardization of our study, none of the study animals died during the study duration. Paracetamol produced significant ($p < 0.05$) elevation in serum bilirubin AST and ALT levels, compared to the toxicant control. Lycopene has caught the attention of investigators as a potential hepatoprotective due to its antioxidant, anti-inflammatory and anti-proliferative properties.[30]

Liver is the chief target organ of lycopene accumulation in the body. After oral administration, lycopene is rapidly absorbed and gets accumulated in the liver, with a lesser amount going to the spleen. Safety of lycopene has been proved beyond doubt in multiple toxicity studies. No significant toxic effects were observed with lycopene up to 2000mg/kg body weight when administered orally. Animals were observed for 24 hours.

There is a need of evaluation of lycopene in other models of hepatotoxicity with higher doses given its wide safety margin. Further studies are essential to expatiate its mechanism of action. In future, lycopene would be a potential hepatoprotective agent against drug induced hepatotoxicity in clinical use. Especially in the prevention/treatment of paracetamol induced hepatotoxicity.[32,33]

CONCLUSION

It is concluded that lycopene emerge hepatoprotective effect against paracetamol induced hepatic damage in rats. Lycopene needs to be evaluated in other models of hepatotoxicity and further studies are required to delineate its mechanism of action. Lycopene might be a potential hepatoprotective for clinical use in future.

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CURCUMIN POTENTIATES THERAPEUTIC EFFICACY OF VOGLIBOSE

Diksha B. Khandale*, Vitthal J. Chaware, Vivekkumar K. Redasani, Ashish T. Thorat

Department of Pharmacology, YSPM's Yashoda Technical Campus Wadhe, Satara, India.

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***Corresponding Author**

Diksha B. Khandale

Department of Pharmacology, YSPM's Yashoda Technical Campus Wadhe, Satara, India.

ABSTRACT

Introduction: Herbal antidiabetic preparations are often used as an add-on therapy in diabetes and such herbal preparations often contain curcumin. Hence, in the present investigation the combine effects of curcumin and voglibose in normal as well as diabetic rats was studied. **Methods:** Streptozotocin (60 mg/kg i.p.) diabetic rats was treated for 14 days with curcumin (30 mg/kg p.o) and voglibose (0.06 mg/kg p.o). After treatments, the blood glucose level was assessed. Data was analyzed using one-way analysis of variance (ANOVA) followed by post hoc Scheffe's test. **Results:** Treatment of diabetic rats with curcumin or voglibose alone decreased the blood glucose level. The combination of voglibose with curcumin further decreased blood glucose levels in diabetic rats, indicating synergestic effect. **Conclusion:** The results highlights that curcumin and voglibose given alone showed effect against STZ induced Hyperglycemia but the combination of curcumin with voglibose showed better and synergistic effect. Therefore, it might be a promising strategy for combating diabetic complications.

KEYWORDS: Hyperglycemia; Curcumin; Voglibose; Streptozotocin; Antidiabetic activity.

1. INTRODUCTION

In today's society, diabetes is a serious metabolic disease. Its frequency has risen considerably in recent years, causing the World Health Organization to classify it as a major public health crisis.[1] The International Diabetes Federation's specialists estimate that 193 million people worldwide have undiagnosed diabetes and are at risk of developing chronic complications. According to estimations, there will be 629 million persons with diabetes worldwide in 2045.[2] It is a chronic metabolic condition characterised by a loss of glucose

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homeostasis as well as changes in lipid and protein metabolism due to abnormalities in insulin secretion, action, or both. Insulin is a protein (hormone) produced by beta cells in the pancreas in response to a variety of stimuli, including glucose, sulphonylureas, and arginine, but glucose is the most important factor.[3]

Analyzing blood sugar levels can be used to diagnose diabetes. On fasting, blood sugar level is 80 mg/dl, and in the postprandial stage, it can reach 160 mg/dl. Finger prick blood sugar test, fasting blood sugar test and glucose tolerance diagnostic test are some of the laboratory tests used to diagnose diabetes.[4]

Impaired insulin secretion, tissue insulin resistance, or a combination of the two are thought to be the most popular factors that contribute to the pathogenic mechanisms of T2DM, a disease spectrum that starts with tissue insulin resistance and progresses to a state marked by complete loss of pancreatic beta cell secretory activity.[5] The fundamental mechanism underlying hyperglycemia involves over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissue.[6]

Streptozotocin (STZ) is a synthetic nitrosoureido glucopyranose derivative isolated from Streptomyces achromogenes fermentations that is utilised as an antitumor antibiotic and is chemically linked to other nitrosureas used in cancer therapy.[7] It produces β-cell toxicity, which leads to insulin insufficiency, and is easily carried into pancreatic β-cells by GLUT-2. The β-cell O-GlcNAcase enzyme, which is in charge of removing O-GlcNAcase from protein, is specifically inhibited by STZ. This leads in β-cell death and irreversible O-glycosylation of intracellular proteins.[8]

When taken alongside standard medications, a combination of herbal pharmaceuticals (or isolated phytochemicals) has been demonstrated to be effective in the treatment of some disorders.[9] Among all of the available medical systems in the world, Indian traditional medicine is one of the most comprehensive.[10]

Turmeric contains curcumin, a yellow pigment derived from *Curcuma longa* that has anticarcinogenic and anti-inflammatory characteristics, including an inhibitory action on TNF-a. Curcumin has also been found to inhibit NO synthesis and scavenge nitrite and peroxynitrite radicals released by macrophages, which helps to lower blood glucose levels and improve the antioxidant capacity of pancreatic β-cells.[11] The anti-inflammatory,

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antioxidant, antiviral, antifibrotic, anticoagulant, and glucose-regulating properties of curcumin are its distinguishing features.[12]

In the treatment of diabetes, alpha-glucosidase inhibitors and fast-acting, short-duration insulin secretagogues are widely utilized. In the final step of carbohydrate digestion, voglibose, alpha -glucosidase inhibitor, reduces the breakdown of disaccharides into monosaccharides by acting competitively on the activities of disaccharidase (alpha -glucosidase). This decreases glucose breakdown and absorption, preventing postprandial hyperglycemia.[13]

As a result, examining these interactions is crucial for illness treatment that is both safe and effective. Despite the fact that Curcumin nanoparticles have been shown to have anti-diabetic properties, the interaction between Curcumin with Voglibose has yet to be studied.

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals

Streptozotocin sterile powder 1gm was purchased from Teva Parenteral Medicines Inc. Irvine.

Curcumin was purchased from Yarrow chem Products Ghatkopar (west) Mumbai, India.

Voglibose was purchased from Discovery Mankind Pharma Ltd. New Delhi.

2.2 Maintenance of animals

Albino rats of Wistar strain weighing 180-200g were used for the studies after obtaining the permission from institutional animal ethical committee. The animals were housed in standard polypropylene cages and maintained under standard laboratory conditions (12 h light/dark cycle; at an ambient temperature of 25 ± 5 °C; 35-60% of relative humidity). The animals were fed with standard rat pellet diet and water *ad libitum.*

2.3 Oral glucose tolerance test

For Oral Glucose Tolerance Test, rats was divided into four groups (n = 6). Medications were given orally to overnight fasted animals.

After half an hour of test medication administration, a glucose solution (2.5g/kg body weight) was given orally in a volume of 1 ml, and blood glucose levels were monitored at 0, 30, 60, 90, and 120 minutes using a Glucopoint glucometer.

2.4 Hypoglycaemic study

For hypoglycaemic study, rats were divided into four groups (n = 6) and were administered vehicle (1ml), curcumin(30mg), voglibose (0.06mg), curcumin(30mg) and voglibose(0.06mg) respectively. The blood glucose levels were estimated on days 0, 7 and 14.

2.5 Induction of Hyperglycemia

Diabetes was induced using streptozotocin (STZ). The animals fasted overnight and diabetes was induced by way of a single intra peritoneal injection of a freshly prepared solution of STZ (60 mg/kg b.w.) in a 0.1 M citrate buffer (pH 4.5). On the third day of STZ-injection, the animals with fasting glycaemia higher than 200 mg/dL and with signs of polyuria and polydipisia were considered to be diabetic and included in the study.

2.6 Experimental design

The diabetic animals, divided into four groups (n = 6) were administered vehicle, curcumin (30 mg/kg), voglibose (0.06 mg/kg), curcumin (30mg) and voglibose (0.06 mg/kg), respectively, for 14 days. The fasting blood glucose levels were estimated on days 0, 7 and 14.

At the end of the treatments, the blood samples were collected for the analysis of blood glucose level. The experimental procedures were approved by the Institutional Animal Ethics Committee.

2.7 Statistical analysis

Using the 7.5 version of SPSS computer programme, data were statistically examined using one way ANOVA, followed by a post hoc Scheffe's test. When the p-value was less than 0.05, the results were considered significant.

3. RESULTS

3.1 Effect of curcumin, voglibose and its combination in oral glucose tolerance test

The curcumin, voglibose and its combination showed a significant reduction in blood glucose levels from 30 min onwards in oral glucose tolerance test as compared to normal group. The results are represented in table 1.

Table 1: Effect of curcumin, voglibose and its combination in oral glucose tolerance test.

Group	Treatment	Blood-Glucose Level (mg/dl)				
		0min	30min	60min	90min	120min
I	Normal Control (Glucose 2.5g/kg)	90.6±9.2	106.7±10.6	157.4±15.7	166.7±16.6	250.1±10.10
II	Curcumin (30mg/kg p.o) + Glucose (2.5g/kg)	83.8±8.3	110.5±11.20	99.8±9.9	94.2±9.4	91.1±9.2
III	Voglibose (0.06mg/kg p.o) + Glucose (2.5g/kg)	82.4±8.4	95.4±9.5	81.2±8.1	80.8±7.9	79.1±7.6
IV	Curcumin (30mg/kg p.o) + voglibose (0.06mg/kg p.o) + Glucose (2.5g/kg)	81.7±7.9	91.7±9.1	89.5±8.7	82.8±8.1	76.7±7.6

Each value represent mean ± S.E.M., *n*=6

3.2 Effect of curcumin, voglibose and its combination in normal animals

In normal animals, curcumin did not significantly reduced blood glucose level on 0th, 7th and 14th day as compared to normal control group. Voglibose significantly reduced blood glucose level on 0th, 7th and 14th day as compared to normal control group. Combination of curcumin and voglibose significantly reduced blood glucose level on 0th, 7th and 14th day as compared to normal control group. The results are represented in table 2.

Table 2: Effect of curcumin, voglibose and its combination in normal animals.

Group	Treatment	Blood-Glucose Level (mg/dl)		
		Day 0	Day 7	Day 14
I	Normal Control	75.4±7.8	75.8±7.8	76.4±7.4
II	Curcumin (30mg/kg p.o)	74.5±7.9	74.6±7.6	73.7±7.3
III	Voglibose (0.06mg/kg p.o)	72.8±7.1	61.9±6.6	61.1±6.2
IV	Curcumin (30mg/kg p.o) + Voglibose (0.06mg/kg p.o)	70.5±6.9	57.9±5.8	53.8±5.3

Each value represent mean ± S.E.M., *n*=6

3.3 Effect of curcumin, voglibose and its combination in diabetic animals

In diabetic animals, curcumin did not significantly reduced blood glucose level on 0th, 7th and 14th day as compared to normal control group. Voglibose significantly reduced blood glucose

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level on 0th, 7th and 14th day as compared to normal control group. Combination of curcumin and voglibose significantly reduced blood glucose level on 0th, 7th and 14th day as compared to normal control group. The results are represented in table 3.

Table 3: Effect of curcumin, voglibose and its combination in diabetic animals.

Group	Treatment	Blood-Glucose Level (mg/dl)		
		Day 0	Day 7	Day 14
I	Diabetic Control STZ (60mg/kg i.p)	381.2±38.1	398.1±39.7	391.7±38.1
II	Curcumin (30mg/kg p.o)	370.5±37.9	360.1±5.1	359.5±5.7
III	Voglibose (0.06mg/kg p.o)	356.1±36.3	149.6±15.1	140.9±14.10
IV	Curcumin (30mg/kg p.o) + Voglibose (0.06mg/kg p.o)	331.2±33.3	116.8±9.66	110.2±12.44

Each value represent mean ± S.E.M., *n*=6

4. DISCUSSION

A series of metabolic illnesses known as diabetes mellitus are characterised by chronic hyperglycemia carried on by deficiencies in insulin secretion, insulin action, or both. The significance of insulin as an anabolic hormone leads to metabolic irregularities in carbohydrates, lipids, and proteins. These metabolic abnormalities are introduced on by insufficient insulin levels to start producing an adequate response and insulin resistance of target tissues, primarily skeletal muscles, adipose tissue, liver, at the level of insulin receptors, signal transduction system, and effector enzymes or genes. The kind and length of diabetes affect the severity of symptoms. Some people with diabetes have no symptoms, especially those who have type 2 diabetes in its early stages. Uncontrolled diabetes may lead to stupor, coma and if not treated death, due to ketoacidosis or rare from nonketotic hyperosmolar syndrome.[14]

Most diabetic complications are caused by persistent hyperglycemia, a typical symptom of diabetes. Treatment should seek to lower blood glucose levels to near-normal levels in all individuals. Oral hypoglycemic medications are currently available for Hyperglycemia treatment. The majority of medications have failed due to ineffectiveness or side effects. There is no cure for diabetes. This problem has highlighted the need for more better, safer, and less expensive diabetes management techniques. Alternative therapies must be discovered in order to solve these challenges and give better therapeutic management. An excellent method to treat hyperglycemia and other DM problems is to combine the actual antidiabetic medications with phytochemicals.[15]

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Streptozotocin is a deoxy-s [(methyl-nitrosoamino) carbonyl)-amino]-D gluco pyranose molecule that causes Hyperglycemia in most laboratory animals. Streptozotocin and other beta cell toxins in high dosages cause insulin insufficiency and Hyperglycemia. Although streptozotocin is favoured because of its more selective beta cell cytotoxicity, its sensitivity varies by species, strain, sex, and nutritional condition, and there are batch variances in activity.[16]

In the search for alternatives to current medication for diabetes mellitus, curcumin has gained attention in the last decade for its antidiabetic properties.[17] Curcumin also reported to have beneficial effects on various diseases, like multiple myeloma, pancreatic cancer, myelodysplastic syndromes, colon cancer, psoriasis, and Alzheimer's disease.[18] In addition, it could delay development of T2DM, improve β-cell functions, prevent β-cell death, and reduce insulin resistance in animals.[19]

Voglibose is alpha -glucosidase inhibitor that also stimulates GLP-1 secretion.[20] Inhibition of enzymes in the digestive organs, such as alpha-glucosidase, can be used to prolong glucose absorption as a treatment for diabetes. Alpha-Glucosidase (α-d-glucoside glucohydrolase) is an exo-type carbohydrase that catalyses the liberation of α-glucose from the non-reducing end of the substrate in microbes, plants, and animal tissues. The blocking of this enzyme decreases the rise in blood sugar after a carbohydrate meal.[21] It slows and reduces the absorption of monosaccharides by preventing the intestinal breakdown of complex carbohydrates into simple sugars.[22]

In our Investigation, the oral glucose tolerance test studies revealed that curcumin, voglibose and combination of curcumin and voglibose has the capacity to lower blood glucose.

Hypoglycemic studies experiments conducted for our investigation showed that curcumin, voglibose, and combinations of curcumin and voglibose had the ability to reduced blood glucose.

In our present antidiabetic study, group II animals treated by curcumin did not significantly reduced blood glucose level. Group III animals treated by voglibose could significantly reduced blood glucose level as compared to toxicant group. Group IV animals treated by its combination could significantly reduced blood glucose level as compared to toxicant group.

5. CONCLUSION

Results obtained from the present study proved that curcumin when given alone did not show effect against STZ induced Hyperglycemia in rats and voglibose when given alone could significantly reduced blood glucose level but more prominent effect was observed when combination of curcumin and voglibose was given. Hence combination of these drugs showed synergestic effect.

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DESIGN, DEVELOPMENT AND EVALUATION OF TRADITIONAL POLYHERBAL FORMULATION TO CURE DENGUE AND CHIKUNGUNYA

Snehal Mahamuni*, Bharatee Chaudhari and Vivekkumar Redasani

YSPM's Yashoda Technical Campus, Faculty of Pharmacy, NH-4, Wadhe, Satara – 415011.

***Corresponding Author: Snehal Mahamuni**

YSPM's Yashoda Technical Campus, Faculty of Pharmacy, NH-4, Wadhe, Satara – 415011.

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ABSTRACT

Herbal medicine is the most traditional method of treatment that has been used throughout history and is utilised in all cultures. The present study was focused on development and evaluation of polyherbal syrup to cure dengue and chikungunya. Dengue and chikungunya viruses are caused by an arbovirus. Both viruses are arthropod-borne viruses sharing a common vector. These two diseases are transmitted from infected person to the healthy person through bite of virus carrying female mosquito. There is no specific treatment for dengue and chikungunya. Generally, the doctors priscribes papaya leaves extract and NSAID drugs to treat the symptoms of dengue and chikungunya. Keeping our hopes up, we took steps to locate a remedy using all natural herbs that have been documented centuries ago and came up with a comparatively safer solution for this ailment. In present research some natural herbs are used to develop safe cost-effective syrup for dengue and chikungunya. The aqueous extracts of selected herbs were formulated in particular ratio to form herbal syrup. The plants chosen were *carica papaya, aloe vera, tinospora cordifolia* and *ocimum tenuiflorum.* The raw materials were collected, authenticated accordance with WHO guidelines. Several experimental batches were created by adjusting the percentage of simple syrup. This batches were tested for a variety of assessment factors. This formulation's accelerated stability was also studied. The formulation complies with all the phytochemical and physicochemical parameters, therefore it is concluded that polyherbal syrup was found to be safe.

KEYWORDS: Dengue, Chikungunya, Polyherbal syrup, Phytochemical screening, Accelerated stability studies.

INTRODUCTION

Dengue and chikungunya are two mosquito-borne viral diseases of great public health concern in India. Dengue virus (DENV) and chikungunya virus (CHIKV) are transmitted by the same species of mosquito, Aedes aegypti and share spatiotemporal territories. DENV belongs to the Flaviviridae family and CHIKV belongs to the genus Alphavirus of Togaviridae.[1] DENV and CHIKV typically incubate for 4-7 days and 3-7 days, respectively. Patients infected with either virus often have an initial onset of fever, myalgia, and headache, with some developing a maculopapular rash and/or gastrointestinal symptoms.[2] Mild dengue fever to severe dengue hemorrhagic fever and/or dengue shock syndrome are the clinical symptoms.[3] The symptoms of dengue are thrombocytopenia, high fever, severe headache, muscle and joint pains, nausea and vomiting, mild pain in throat and extreme weakness.[4] Chikungunya virus (CHIKV) is an arbovirus spread by mosquitos. When symptomatic (85 to 95% of cases), CHIKV infection causes an acute fever–arthralgia syndrome that can evolve into chronic inflammatory rheumatism.[3] The symptoms of chikungunya are polyarthralgia, sudden high fever, joint pains and muscle pains, diarrhoea, abdominal pain and fatigue.[5]

Till date, there are no specific globally accepted treatments for dengue fever and chikungunya fever in any system of medicine. DENV and CHIKV does not cause very high mortality. Traditionally large numbers of plants are reported for their use against contagious diseases, including infection caused by viruses.[6] During the critical phase of dengue, malaria, chikungunya that thrombocytopenia is characterized by a decrease in platelet count below 100000 perm3 from the baseline.[7]

Herbal formulation

Herbal medicine are treated as traditional medicines since they were extensively used in traditional system of medicine like Ayurveda, siddha, Unani.[8] The majority of herbal syrup was obtained from plants, and herbal medicine refers to the use of extract for therapeutic purposes. Herbal medications are also available in syrup form, in addition to conventional dosage forms.[9]

Following are the ingredients used in formulation

1. **Papaya leaves:** *Carica papaya* (papaya, paptia, paw paw) is an herbaceous plant belonging to the family

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Caricaceae. Tradtionally the *Carica papaya* leaf extract used in dengue and chikungunya fever patients with thrombocytopenia; it accelerates the increase in the platelet count.[10,11,12]

2. **Aloe vera:** It consist of fresh leaves of *Aloe barbadensis* belonging to family *Liliaceae*. Aloe vera reduces inflammation and arthritis caused by adjuvants.[13,14,15]
3. **Gulvel:** *Tinospora cordifolia*, also known as "Guduchi" in Sanskrit. It is a significant medicinal herb used in Ayurvedic medicine to treat polyarthralgia, osteoporosis, colds, fevers, diabetes, and even rheumatoid arthritis.[16,17,18,19,20]
4. **Tulsi:** Tulsi is an aromatic plant belonging to the family *Lamiaceae*. It is useful in dengue, chikungunya and malarial fever.[21,22,23,24]

MATERIALS AND METHODS

Collection and Authentication of plant material

Leaves of *Carica papaya, Aloe barbadensis, Tinospora cordifolia, Ocimum tenuiflorum* were collected from the herbal supplier. All the plant material were authenticated by Y. C. College, Department of Botany, Satara.

Preparation and Phytochemical evaluation of extracts

The plant material was washed thoroughly with running tap water, more than five times. The main stems of the leaves was removed using a scissor. The material was cut in to pieces and washed it well with boiled cool water. Choped into even smaller pieces. The pieces were grinded well for about 15 minutes with 50mL boiled cold water till a uniform pulp is formed. The pulp was placed into the juice extractor and squeezed it till get the pure extract. The phytochemical evaluation of extracts was done individually.

Preparation of simple syrup

The 66.67gm of sugar was weighed and added to purified water and heated until it dissolve with occasional stirring. Sufficient boiling water was added upto 100ml.

Preparation and Phytochemical evaluation of polyherbal syrup

The simple syrup (66.67%w/v) was prepared as per Indian pharmacopoeia. The extracts were added into simple syrup I.P. and the volume was made upto 100ml. The 3 trial batches were prepared by varying the concentration of simple syrup.

Formulation table

Composition of polyherbal syrup

Ingredients	Quantity			Activity
	A	B	C	
Papaya Leaf Juice	25 ml	25 ml	25 ml	Platelet increasing agent
Aloe vera Juice	18 ml	18 ml	18 ml	Anti-inflammatory agent
Gulvel Juice	8 ml	8 ml	8 ml	Anti-arthritic agent
Tulsi Juice	10 ml	10 ml	10 ml	Analgesic agent
Simple Syrup	46.66%	56.66%	66.66%	Base, Viacosity modifies

Evaluation parameters

a. Colour, Odour, Taste

The syrup's colour, odour and taste were examined.[25]

b. Determination of pH

The 10ml of final syrup was taken in to the volumetric flask and filled the volume upto 100ml with distilled water. The pH paper was used to measure the Ph.[25,26]

c. Determination of viscosity

The viscosity of syrup was measured using an ostwald viscometer. Firstly the ostwald viscometer was cleaned with warm chromic acid or acetone. The water was filled up to the mark "G" in the dry viscometer and placed the viscometer vertically on a suitable platform. The time was noted while water was flowing from mark A to mark B. This operation was done at least three times and recorded the time to acquire reliable readings. Then the viscometer was rinsed and filled it with test liquid (syup) till mark A, then the time was calculated to takes for the liquid to flow up to mark B.[26]

Formula for viscosity

$$\text{Viscosity} = \frac{\text{Density of test liquid} \times \text{Time required to flow test liquid} \times \text{Viscosity of water}}{\text{Density of water} \times \text{time required to flow water}}$$

d. Determination of density

The density of syrup was calculated using the beaker. The beaker was cleaned with chromic acid or nitric acid. The beaker was rinsed two to three times with distilled water. The weight of the empty dry beaker was noted. The beaker was filled with test liquid, and the excess liquid out of the beaker was wiped. Finally, calculated the weight in grams of a liquid.[9]

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Formula for density

$$\text{Density of liquid under test (syrup)} = \frac{\text{Weight of liquid under test}}{\text{Volume of liquid under test}}$$

e. Determination of specific gravity

After cleaning with chromic acid or nitric acid, the bottle was rinsed with filtered water two to three times. The weight of an empty dry bottle was taken with a capillary tube stopper (w1). The bottle was filled with distilled water, screw on the stopper, and the liquid from the outside of the bottle was wiped. And using an analytical balance, weighed the bottle with distilled water (w2). After emptying and drying, the procedure was repeated by replacing water with the liquid under test (syrup). The container was weighed with the stopper and the liquid under test on an analytical balance (w3).[9,27]

Formula for specific gravity

$$\text{Specific gravity of liquid} = \frac{\text{Weight of liquid under test}}{\text{Weight of water.}} \text{ under test (syrup)}$$

Accelerated stability study

Based on the results, the trial batch C was chosen as the most acceptable normal range of parameters. The produced polyherbal syrup was evaluated to an accelerated stability investigation for three months. The syrup was maintained at room temperature and was kept in an amber-colored container. Every month, pH, viscosity, density, and specific gravity were measured. The phytochemical analysis was done at the end of every month.[28]

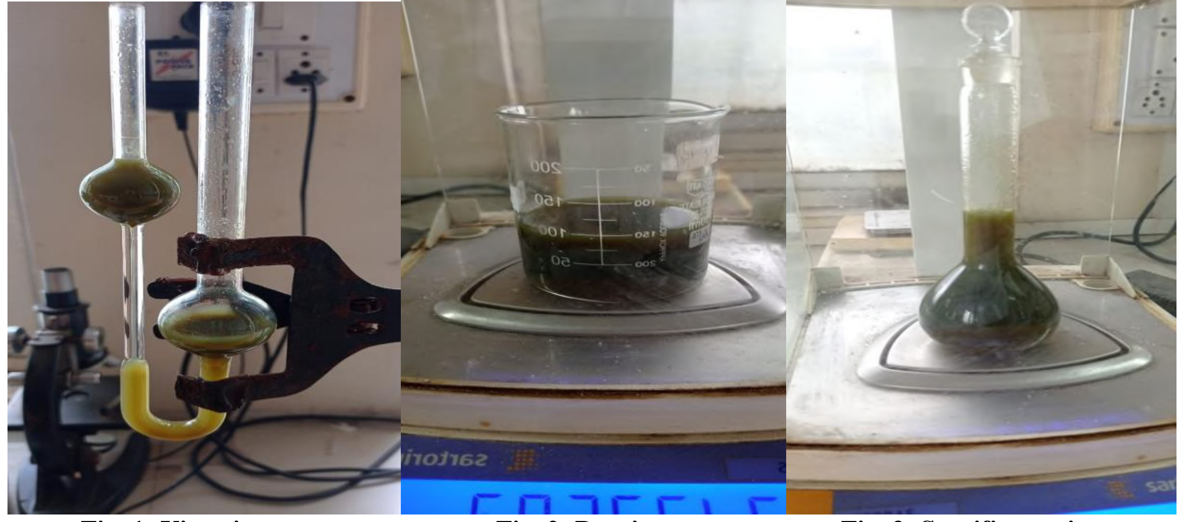

Fig. 1: Viscosity. **Fig. 2: Density.** **Fig. 3: Specific gravity.**

RESULT AND DISCUSSION

Raw material analysis

The phytochemical evaluation of individual herbs are given in table:

Components	Papaya	Aloe vera	Gulvel	Tulsi
Alkaloid	+	+	+	+
Flavonoid	+	+	+	+
Tannin	+	+	+	+
Saponin	+	+	-	+
Phenol	+	-	-	+
Carbohydrate	-	+	+	-
Glycoside	+	-	+	-
Terpenoid	-	-	-	+
Protein	+	+	+	+
Steroid	+	-	+	+

Evaluation of trial batches

The trial batches were evaluated for physical parameters such as colour, odour, taste, pH, viscosity, density and specific

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gravity etc. The results are given in table:

Sr. No.	Parameters	Batch A	Batch B	Batch C
1	Colour	Greenish Brown	Greenish Brown	Greenish Brown
2	Odour	Characteristic	Characteristic	Characteristic
3	Taste	Bitter	Bitter	Bitter
4	pH	6	6.1	6.2
5	Viscosity	0.0153	0.01625	0.018
6	Density	1.05	1.06	1.07
7	Specific gravity	1.432	1.424	1.424

Based on the results, the trial batch C was chosen as the most acceptable normal range of parameters.

Phytochemical analysis of polyherbal syrup for batch C

Chemical constituents	Results
Alkaloid	+
Flavonoid	+
Tannin	+
Saponin	+
Phenol	+
Carbohydrate	+
Glycoside	+
Terpenoid	+
Protein	+
Steroid	+

Accelerated stability study for batch C

Physical Parameters of Polyherbal syrup:

Sr. No.	Parameters	Initial study	First month	Second month	Third month
1.	Colour	Greenish Brown	Greenish Brown	Greenish Brown	Greenish Brown
2.	Odour	Characteristic	Characteristic	Characteristic	Characteristic
3.	Taste	Bitter	Bitter	Bitter	Bitter
4.	pH	6.2±0.03	6.0±0.02	6.1±0.02	6.1±0.04
5.	Viscosity	0.018±0.03	0.018±0.03	0.017±0.04	0.016±0.05
6.	Density	1.06±0.05	1.07±0.04	1.07±0.03	1.06±0.06
7.	Specific gravity	1.424±0.01	1.432±0.02	1.424±0.02	1.433±0.03

Phytochemical analysis of polyherbal syrup for batch C

Chemical constituents	Initial study	First month	Second month	Third month
Alkaloid	+	+	+	+
Flavonoid	+	+	+	+
Tannin	+	+	+	+
Saponin	+	+	+	+
Phenol	+	+	+	+
Carbohydrate	+	+	+	+
Glycoside	+	+	+	+
Terpenoid	+	+	+	+
Protein	+	+	+	+
Steroid	+	+	+	+

CONCLUSION

The polyherbal syrup consisting of four herbs which folklore claim of being used in dengue and chikungunya and these were evaluated and standardized. Also the physicochemical properties of prepared syrup like colour, odour, taste, pH, viscosity, density and specific gravity were satisfactory and the formulation was within the all specification. The phytochemical analysis was done of prepared syrup for three months. The accelerated stability study for three months indicates that the formulation is stable under room temperature.

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Evaluation of protective role of a Ferulic acid on Letrozole induced polycystic ovarian syndrome in female rats

Karishma M. Yadav[1]*, Priyanka K. Ghadage[1], Rupali V. Bhoite[1], Prajakta B. Phadtare[1], Omkar A. Devade[2]

[1]Department of Pharmacology, YSPM's Yashoda Technical Campus, Wadhe, Satara, India.
[2]Department of Pharmacology, AISSMS College of Pharmacy, Pune - 411001, India.

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ABSTRACT: Background: *Ferulic* (hydroxycinnamic) *acid* is an antioxidant of phenolic phytochemical group used for the skin care product. Polycystic Ovarian Syndrome (PCOS) is a state of hormonal disorder causing an enlarged ovary with small cysts at the outer edges. Aim: The study was designed to investigate the protective effect of ferulic acid (3-methoxy-4-hydroxycinnamic acid) in letrozole induced polycystic ovarian syndrome in rats (PCOS). Methods: All the experimental animals except control group were orally administered with Letrozole (1mg/kg) dissolved in 0.5 % w/v Carboxymethyl cellulose (CMC) solution per oral route for 21 days to induce PCOS. Followed by a dose of ferulic acid (10, 20, and 40 mg/kg p.o.) for 15 days using water as vehicle. Results: The PCOS was confirmed in the letrozole induced rats with increased concentration of androgen, abnormal lipid levels, glucose, glycosylated haemoglobin and also depletion of antioxidants. The administrated of letrozole cause to abnormalities in serum hormone profile, lipid profile, blood glucose levels and increases body weight and ovary weight. Ferulic acid successfully exerted its protective effect by restoring all the parameters to normalize and improving or disappearance of ovarian cysts. Histopathological observations showed a remarkable recovery of the ovarian tissue and the presence of normalized structure of antral follicle. Conclusion: Ferulic acid showed protective effects in letrozole induced PCOS in rats. Biological effects of ferulic acid make it a promising drug for treating clinical and pathological abnormalities against PCOS conditions.

Corresponding author
Ms. Karishma M. Yadav,
YSPM's Yashoda Technical Campus,
Wadhe, Satara, India.
Tel: +91-8767445487
E. Mail ID: y.karishma53@gmail.com

Keywords: PCOS, Fertility; Ovulation, Letrozole, Ferulic acid, Cysts.

INTRODUCTION:

Polycystic ovary syndrome (PCOS) is a common and complex female endocrine disorder in women of reproductive age [1,2] with an estimated prevalence of 6 to 10 % [3]. Clinical manifestation of PCOS amenorrhea, abdominal obesity, hirsutism, and androgen excess (Hyperandrogenism), infertility, and expanded ovaries with multiple cysts. Women with PCOS are at increased risk for diabetes, dyslipidemia, atherosclerosis,

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bleeding, hypertension, cardiovascular disease as well as endometrial carcinoma [4]. It is also related with psychological impairments like depression and related mood disorders.

Lipid imbalance, insulin resistance, oxidative stress, and genetics are some of the contributing factors of PCOS [5]. Currently, many therapies are available to induce ovulation and manage PCOS, but it is associated with mild to severe side effects, like; arthritis, hot flushes, muscle or joint pain and psychological side effects like, mood swings, depression, irritability, and bloating. Therefore now-a-days focus is being laid on natural source herbal medicinal plants that have been utilized for the treatment of the various disorders related to the reproductive system due to the lesser or no side effects [3].

Ferulic acid(2*E*)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid) is water soluble, phenolic compound found in active chemical constituent in Chinese medicine herbs such as female ginseng ,and many staple foods, like; fruits, cereals, vegetables and coffee [6,7]. Ferulic acid has been reported to possess a wide variety of biological effects like Antioxidant, anti-inflammatory, hypoglycaemic, and Hyperlipidemic activities [8]. In this study we evaluated that Ferulic acid (3-methoxy-4-hydroxycinnamic acid) may be beneficial in management of PCOS induced by Letrozole due to the reported activity.

MATERIALS:

Drugs and reagents:

Letrozole and Clomiphene citrate were purchased from retail Shop Satara, India. Ferulic acid was obtained from Dolphin Pharmacy Instruments, Pvt., Ltd. Mumbai.

METHODS:

In this study the experimental models used is Letrozole induced PCOS models. The model was widely used accepted for assessing PCOS activity. All animals were selected and divided into six groups and housed eight female rats per cage. All animals in five groups except control group were orally administered with Letrozole for 21 days.

Two animals from each group were scarified by using CO_2 chamber. Ovaries was removed and observed for presence of cysts. On 22nd day, Test group I, II, and III was administered with Ferulic acid for 15 days, whereas standard group was dosed with Clomiphene citrate for 15 days per oral route [9-11].

Animals:

This prospective comparative study was conducted at Department of Pharmacology, YSPM's Yashoda Technical Campus, Wadhe, Satara, and Maharashtra, India. Healthy, Virgin, cyclic and adult female wistar rats (150 to 200 g) were used in the present study. These animals were procured from registered breeder and acquainted in the quarantine area for one week.

Housing of animals:

The animals were housed in polypropylene cages with paddy husk as bedding. The animals were maintained under standard laboratory conditions of 22 ± 2°C temperatures, 50 ± 15 % of relative humidity, 12 h dark/ 12 h light cycle with free access to pellet diet and water provided *ad libitum*. The study protocol was approved form institutional animal ethic committee. The experiments were performed as per as guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Governments of India. The Institutional Animal Ethics Committee approved the study protocol YSPM/YTC/PHARMA-IAEC/48/2020.

PCOS induction:

All the experimental animals except control group were orally administered with letrozole (1 mg/kg) dissolved in 0.5 % w/v CMC solution per oral route for 21 days to induce PCOS. Vaginal smear checked or examined daily and the animals in regular estrous phase were selected for study. Vaginal smears were collected and evaluated microscopically using Crystal violet stain to confirm the induction of PCOS. Two animals from each group were scarified by using CO_2 chamber. Ovaries were removed and observed for presence of cysts [11,12]. In female rats, the estrous cycle characterized by proestrus, estrus, metestrus (or diestrus I) and diestrus (or diestrusII) in normal animals. During estrus cyclic differences in vaginal cytology occurs in response to the morphological changes and continuous changes in cell types (leukocytes, nucleated epithelial and cornified epithelial) occurs in PCOS induced animals [8,9].

Treatment groups:

Animals were randomly assigned into six group (Table 1) and adequate supply food and drinking water.

Study design:

The study consisted of 48 female Albino Wistar rats equally divided into 6 groups as group 1 (control

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group), group 2 (PCOS induced group), group 3 (Standard group), group 4, 5, and 6 as treatment groups. Following Letrozole administration, standard group was administered with Clomiphene citrate at a dose of 1mg/kg in 0.5 % CMC per oral and treatment group 4, 5, and 6 were administered Ferulic acid with the dose of 10, 20, and 40 mg/kg of body weight respectively in water per oral for 15 days. After 21 days, PCOS control group and after 36 days, animals from other groups were fasted overnight and blood was collected by retro orbital puncture then serum was separated and was used for estimation of hormones, lipid parameters and glucose. Body weight was measured at the end of study (On day 36th) animals were then sacrificed and ovaries were excised, cleaned of fat and weighed [11].

Table 1. Treatment Groups.

Group 1: Control	Healthy rats were administered vehicle (10 ml/kg)
Group 2: Negative control	Animals were administered with Letrozole (1 mg/kg)
Group 3: Positive control	Animals were administered with Letrozole (1 mg/kg) + Clomiphene citrate (1 mg/kg)
Group 4: Test group with low dose	Animals were administered with Letrozole (1 mg/kg) + Ferulic acid (10 mg/kg)
Group 5: Test group with intermediate dose	Animals were administered with Letrozole (1 mg/kg) + Ferulic acid (20 mg/kg)
Group 6: Test group with high dose	Animals were administered with Letrozole (1 mg/kg) + Ferulic acid (40 mg/kg)

Biochemical estimation:

***Measurement of fasting blood glucose*:**

Blood glucose level was measured by using Accu-cheak active glucometer (Roche Diabetes care GmbH Sandhofer Strasse11668305 Mannheim, Germany).

***Hormonal assay*:**

Blood samples were collected by retro-orbital puncture; serum was used for hormonal estimation (FSH, LH and Testosterone). Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), Testosterone was measured via Enzyme Linked Immunosorbent Assay (ELISA) with the help of commercial kits (ELISA kit).

***Lipid profile*:**

The lipid profile (LDL, HDL, Total cholesterol, Triglycerides) was estimated at the end of the study. Lipid profile (LDL, HDL, Total cholesterol, Triglycerides) were quantified by using enzymatic kits procured from Aspen Laboratories pvt, Ltd

***Histopathology*:**

The excised ovaries were fixed in 10 % v/v formalin solution. According to histological procedure, they were subjected to tissue processing by washing with water which was followed by dehydration through ascending grades of alcohol then cleared through xylene. Then paraffin embedding method was used. The blocks were sectioned by using microtome and were placed on slides. These sections were stained with hematoxylene-eosin (HE), dehydrate, cleared and mounted on DPX mount under glass cover slips. The light microscope was used for observation which was connected to a camera to capture image.

Statistical analysis:

The statistical analysis was done by using Graph pad software version 5.0 and results were compared by one-way ANOVA followed by Tukey's Multiple Comparison Test. The results were analysed by Two-way analysis of variance followed by Bonferroni posttests. A p value <0.05 was considered as statistically significant.

RESULTS:

Examination of oestrus cycle:

Fig 1. showed oestrus cycle phase of animals. Displaying oestrous cycle stage only animals with a regular cycle were used for research, Fig 2 demonstrated not observed cornified squamous epithelial cells (Crystal violet staining) in PCOS induced groups.

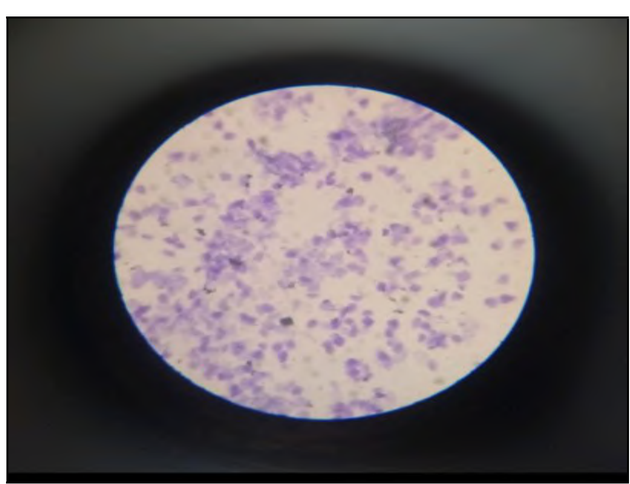

Fig 1. Smear with cornified squamous epithelial cells (Normal animals).

Showed oestrus cycle phase of animals. Displaying oestrous cycle stage only animals with a regular cycle were used for research.

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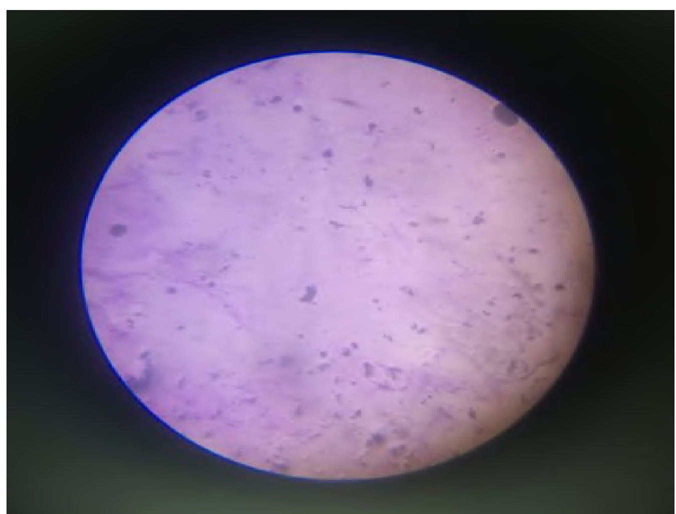

Fig 2. Examination of oestrus cycle (PCOS induced animals).
Not observed cornified squamous epithelial cells (Crystal violet staining) in PCOS induced groups.

Morphology of ovary:
Fig 3 shows Normal ovary structure, where as Fig 4 shows Fluid filled cysts in PCOS induced group.

Fig 3. Morphology of ovary (Normal ovary).

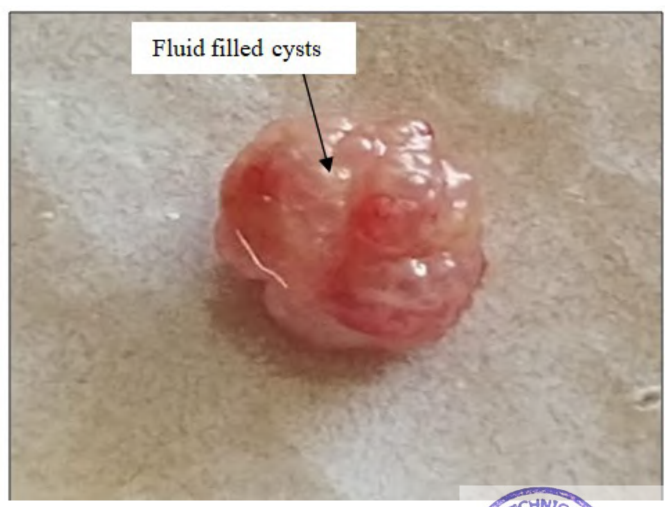

Fig 4. Morphology of ovary (Fluid filled cysts in PCOS induced group).

Body weight:
The effect of Ferulic acid on body weight was represented in Fig 5. Letrozole treatment to a significantly increase in body weight ($p<0.001$) as compared to control group. Oral treatment with Ferulic acid at dose of 10, 20, 40 mg/kg, for 2 weeks ($P<0.001$, $P<0.001$ and $P<0.001$; respectively) significantly reduced the body weight in experimental animals while treatment with Clomiphene citrate (1 mg/kg) significantly decreased ($P<0.001$) body weight when compared to Negative control rats.

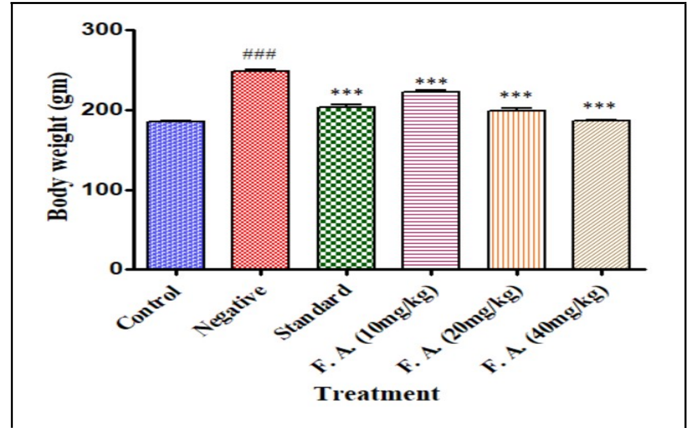

Fig 5. The effect of Ferulic acid on body weight.
All values represent mean ±SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. ###p<0.001; when compared with normal control. ***p<0.001; when compared with negative control.

Organ weight:
Letrozole treatment to a significantly increase in ovarian weight ($p<0.001$) as compared to control group. Oral treatment with Ferulic acid at dose of 10, 20, 40 mg/kg, for 2 weeks ($P<0.01$, $P<0.001$ and $P<0.001$; respectively) significantly reduced the ovary weight in experimental animals while treatment with Clomiphene citrate (1 mg/kg) significantly decrease ($P<0.001$) ovary weight when compared to Negative control rats as given in Fig 6.

Serum hormonal profile:
The serum levels of Testosterone and luteinizing hormone (LH) were increased in PCOS induced group ($p<0.001$, $p<0.001$; respectively) while follicle stimulating hormone significantly decreased ($p<0.001$) in comparison to the control group. A significant fall ($p<0.001$) in testosterone levels was observed in standard, low dose, intermediate dose and high dose groups. Treatment with at dose of Ferulic acid 10, 20, 40 mg/kg and standard ($P<0.01$, $p<0.01$, $p<0.001$, and

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Table 2. The effect of Ferulic acid on serum hormonal level.

Groups	Testosterone (ng/ml)	LH (ng/ml)	FSH (ng/ml)
Control	0.092 ± 0.003	12.17 ± 0.70	25.67 ± 2.72
Negative	0.140 ± 0.003###	19.33 ± 1.25###	10.50 ± 0.99###
Standard	0.112 ± 0.001***	11.17 ± 0.60***	21.67 ± 0.80***
F. A. (10 mg/kg)	0.119 ± 0.002***	15.0 ± 0.68**	15.33 ± 1.11
F. A. (20 mg/kg)	0.092 ± 0.002***	14.50 ± 0.76**	17.50 ± 0.99*
F. A. (40 mg/kg)	0.083 ± 0.002***	11.17± 0.60***	20.17 ± 0.60***

Note: All values represent mean ±SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. ###p<0.001; when compared with normal control. *p<0.05, **p<0.01, ***p<0.001; when compared with negative control. LH and FSH are luteinizing and follicular stimulating hormone.

Table 3. The effect of Ferulic acid on lipid profile.

Groups	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Triglyceride (mg/dL)
Control	61 ± 1.65	26 ± 1.18	22.17 ± 1.30	82.50 ± 1.97
Negative	102 ± 2.58###	14.67 ± 0.66###	51.17 ± 2.10###	132.80 ± 2.82###
Standard	76.67 ± 1.74***	22.67 ± 0.88***	38.67 ± 0.88***	90.83 ± 2.57***
F. A. (10mg/kg)	90.67 ± 1.97**	19.17 ± 1.07*	41.50 ± 0.76**	109.70 ± 2.48***
F. A. (20mg/kg)	71.17 ± 1.35***	21.50 ± 0.76***	37.17 ± 1.32***	90.67 ± 1.97***
F. A. (40mg/kg)	62.50 ± 1.89***	27.67 ± 0.88***	26.67 ± 2.33***	75.67 ± 2.96***

Note: All values represent mean ±SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. ###p<0.001; when compared with normal control. *p<0.05, **p<0.01, ***p<0.001; when compared with negative control.

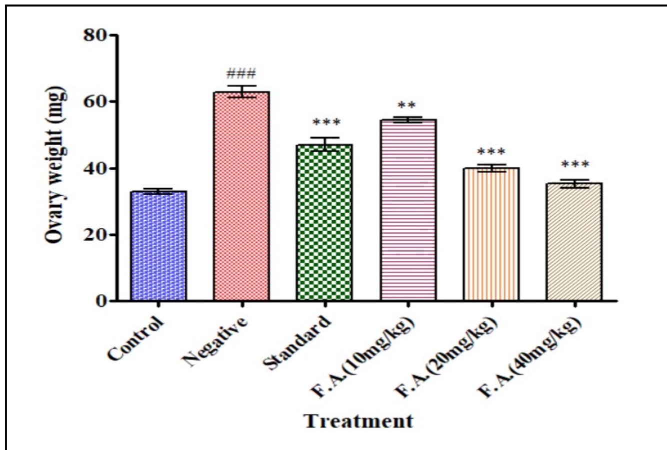

Fig 6. The effect of Ferulic acid on ovarian weight.
All values represent mean ±SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. ###p<0.001; when compared with normal control. **p<0.01, ***p<0.001; when compared with negative control.

p<0.001; respectively) produced a significant decreased in Luteinizing hormone levels when compared with Negative group. Animals treated with at dose of Ferulic acid 20, 40 mg/kg and standard produced a significant increase (p<0.05, p<0.05, and P<0.001; respectively) in FSH levels when compared with Negative group (Table 2).

Ferulic acid reduces blood glucose level:

The effect of Ferulic acid on blood glucose levels was represented in Fig 7. Letrozole treatment to a significantly increase in blood glucose levels (p<0.001) as compared to control group. Oral treatment with at dose of Ferulic acid 10, 20, 40 mg/kg, for 2 weeks (P<0.001, P<0.001 and P<0.001; respectively) significantly decreased the blood glucose levels in experimental animals while treatment with Clomiphene citrate (1mg/kg) significantly decrease (P<0.001) blood glucose levels when compared to Negative control rats.

Lipid profile:

The effect of Ferulic acid on serum lipid profile was represented in Table 3. Letrozole treatment showed

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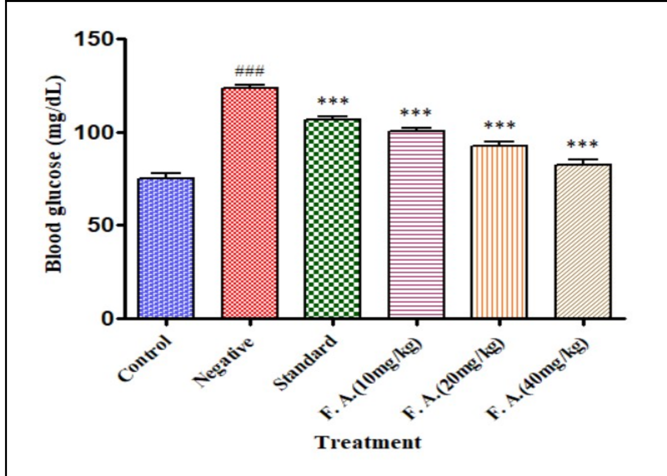

Fig 7. Ferulic acid reduces blood glucose level.
Note: All values represent mean ±SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. ###p<0.001; when compared with normal control. ***p<0.001; when compared with negative control.

Lipid profile:

The effect of Ferulic acid on serum lipid profile was represented in Table 3. Letrozole treatment showed significant changes in serum lipid as compared to control. Cholesterol, LDL and triglyceride were greatly increased as p<0.001, p<0.001 and p<0.001 respectively while HDL levels were decreased (p<0.001) in PCOS induced group (Negative group). Clomiphene treatment significantly decreased Cholesterol (p<0.001), LDL (p<0.001) and triglyceride (p<0.001) levels when compared to PCOS induced group. While HDL levels significantly increased (p<0.001) when compared to PCOS induced group. Low dose of Ferulic acid (10 mg/kg) decreased the levels of Cholesterol (p<0.01), LDL (p<0.01) and triglyceride (p<0.001). It also increased HDL level significantly (p<0.05) in comparison to negative group. Intermediate dose of Ferulic acid (20 mg/kg) decreased the levels of Cholesterol (p<0.001), LDL (p<0.001) and triglyceride (p<0.001). It also increased HDL level significantly (p<0.001) in comparison to negative group. High dose of Ferulic acid (40 mg/kg) decreased the levels of Cholesterol (p<0.001), LDL (p<0.001) and triglyceride (p<0.001). It also increased HDL level significantly (p<0.001) in comparison to negative group.

Histomorphological changes

Histopathological examination of stained sections of ovary showed ovarian changes and ovarian follicular cysts (Fig 8). Yellow coloured arrow showing numbers of ovarian follicular cysts. Negative group showing multiple numbers of ovarian follicular cysts compared to normal control group. Oral administration of Clomiphene citrate (1 mg/kg), low dose of Ferulic acid (10 mg/kg), Intermediate dose of Ferulic acid (20 mg/kg), and high dose of Ferulic acid (40 mg/kg) significantly improved or disappearance the number of ovarian follicular cysts compared to negative group.

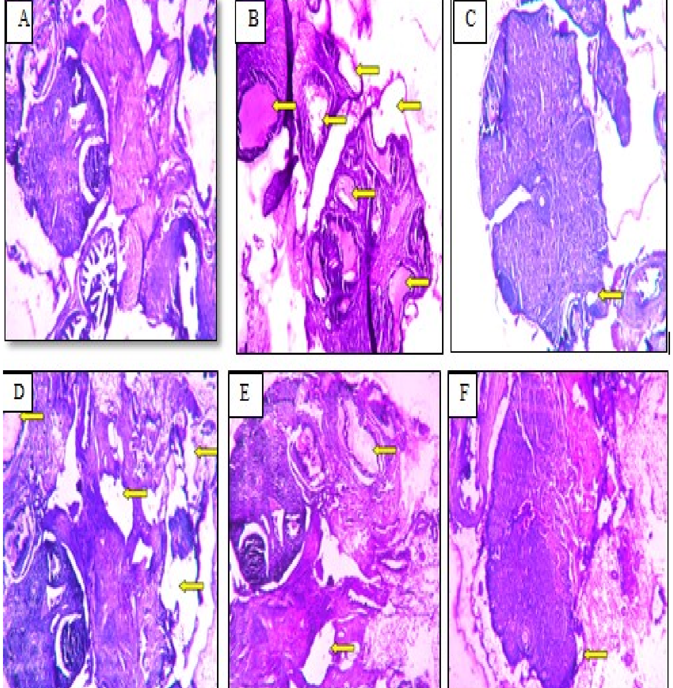

Fig 8. Effect of Ferulic acid in HE-stained ovary tissue (40X).
A. Normal control: showing normal histology of ovary. B. PCOS control: showing large numbers of ovarian follicular cysts. Yellow arrow indicates cysts. C. Letrozole + Clomiphene citrate showing less numbers of cysts. Yellow arrow indicates cysts. D. Letrozole + Ferulic acid (10 mg/kg) showing fewer moderate numbers of cysts. Yellow arrow indicates cysts. E. Letrozole + Ferulic acid (20 mg/kg) showing less numbers of cysts. Yellow arrow indicates cysts. F. Letrozole + Ferulic acid (40 mg/kg) showing less numbers of cysts. Yellow arrow indicates cysts.

DISCUSSION:

Polycystic ovarian syndrome (PCOS) is major female health problem. It is a chronic metabolic disorder characterized by hyperglycaemia, obesity, excess androgen level, hyperlipidaemia, and decrease FSH level. The World Health Organization estimates that it affects 116 million women worldwide as of 2012 [13]. Various experimental models for PCOS have been developed in rats like administration of testosterone propionate (TP), dehydroepiandrosterone (DHEA), and 5a-dihydrotestoterone (DHT) and Estradiol valerate (EV). It is models fully convincing and identify with the

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condition of human PCOS completely [14]. Letrozole is a non-steroidal aromatase inhibitor that reduces conversion of androgens to estrogens in the ovary, resulting increased testosterone and decreased E2 production and stimulate PCOS like condition by causing circulating hyperandrogenism, hormonal imbalance, and intra ovarian androgen excess leading to appearance of polycystic ovary. Letrozole induced PCOS was causehyperglycaemic condition which may contribute to insulin resistance, hyperlipidaemia leading to metabolic syndrome [10-15]. Letrozole induce animal model causes polycystic ovarian syndrome in our research study. It is PCOS rat model characterized by an increase in androgen biosynthesis. P450 aromatase enzyme is responsible converting testosterone and androstenedione to estradiol and estrone. This enzyme inhibits activity led to enhance ovarian androgen production or concentration and resulted in PCOS disorder. Due to inhibit of aromatase enzyme activity increases ovarian androgen secretion and resulted into increase level or concentration of testosterone, LH, and FSH, Letrozole treatment showed some metabolic feature, like increased body fat, triglycerides, cholesterol and body weight [10,14]. Ferulic acid showed marked significantly decreased body weight and ovary weight in PCOS rats that may be responsible for reduced the fatty formation, decreasing follicular cysts (follicular fluid). The body weight was considerably reduced by treatment with Ferulic acid (20 and 40 mg/kg). The weight of ovaries in the negative control group was greater than that of normal control group rats. Ferulic acid (20 and 40 mg/kg) treatment significantly decreased ovaries weights which matched to those in control group animals. Type-2 diabetic mellitus and insulin resistant hyperglycaemia are inter-linked with PCOS. Altered insulin levels which can directly stimulate ovarian androgen production in PCOS Insulin stimulate adrenal steroidogenesis by enhancing sensitivity to adrenocorticotrophic hormone (ACTH) and increase pituitary LH release. Increase androgen level cause ovarian cyst. FA improves altered insulin levels, impaired glucose homeostasis and insulin sensitivity [15]. PCOS induced rats showed marked rise in blood glucose level relative to control group. Oral administration of Ferulic acid significantly reduced the increased blood sugar levels, and indicating the beneficial impact of Ferulic acid on insulin resistance and diabetic condition. Women with PCOS are hyperandrogenemic which is associated with alteration in circulating lipoprotein and lipid level resulting in dyslipidemia. Regulation of carbohydrate metabolism, insulin plays important role in the metabolism of lipids. Insulin is inhibitor of lipolysis, since it inhibits the activity of the hormone-sensitive lipases in adipose tissue and increased FFA concentration into the circulation. Increased FFA concentration also raises β-oxidation of fatty acids, producing more acetyl-CoA and cholesterol. FA decreased the levels of FFA, TG, Cholesterol and phospholipids in plasma [16-19]. Characteristically PCOS patient have increased cholesterol level. The women with PCOS tend to be obese probably due to high cholesterol and lipid content. The same effect was seen in current research work after PCOS induction. In comparison with the normal control group, the negative control group reported significantly enhanced LDL, Cholesterol, triglycerides concentration and lowered HDL concentration. Ferulic acid (10, 20, and 40 mg/kg) decreased significantly LDL, cholesterol, triglycerides levels and enhanced HDL level. Ferulic acid displayed beneficial outcome against hyperlipidaemia. In this research, non-steroidal aromatase inhibitor Letrozole blocks the conversion of testosterone to estradiol. This lead in testosterone and LH level increased while FSH level decreased. This imbalanced hormonal level leads to inconsistent oestrus cycle [20,21]. The similar condition has been noted in our research. Letrozole induced rats showed considerably increased levels of testosterone, LH and decreased FSH levels compared to control. Standard drug Clomiphene citrate (1 mg/kg), and Ferulic acid (20 and 40 mg/kg) treated rats showed significantly decreased testosterone, LH level and FSH level increased. The Histopathological report of Letrozole induced rats indicated the existence of polycysts in the ovary. Negative group showed large numbers of ovarian follicular cysts. After treatment with Ferulic acid (20 and 40 mg/kg), decreased or improved numbers of ovarian follicular cysts. All the biochemical and Histopathological parameters in our results advocate the Ferulic acid is most constructive treatment against PCOS.

CONCLUSION:

Treating the various parameters in PCOS induced rats, the impact of Ferulic acid treatment with intermediate (20 mg/kg) and high (40 mg/kg) dose was observed to be similar with standard treatment (Clomiphene citrate). In Letrozole induced PCOS animals, Ferulic acid restored the lipid profile, hormone and glycemic status

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as well as ovarian morphology. Ferulic acid might be beneficial in managing PCOS condition due to multiple pharmacological actions like hypoglycemic effects, antihyperlipidemic, anti-inflammatory, protective action against obesity, phytoestrogenic and antioxidant activity. Biological effects of Ferulic acid make it a promising drug for treating clinical and pathological abnormalities against PCOS condition.

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REVIEW ARTICLE

A Review on *in situ* Gel of Gastro Retentive Drug Delivery System

Bhagyashri V. Aiwale*, Bharatee P. Chaudhari, Atish B. Velhal, Vivekkumar K. Redasani
YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara, India.
*Corresponding Author E-mail: **bhagyashriaiwale1998@gmail.com**

ABSTRACT:
The 'in situ gel' system has appeared as one of the most effective drug delivery systems. Its specific distinguishing feature of 'Sol to Gel' transition aids in the continuous and controlled release of medicines. These systems have a number of advantages, including simple production, convenience of use, enhanced adherence, and patient comfort by lowering drug delivery frequency due to their distinctive sol to gel transition characteristics. An in-situ gelling system is a formulation that is in solution form before penetrating the body but transforms to gel form under specified physiological conditions. This review mainly focused on introduction, Advantages and Disadvantages of in situ gel, mechanism, types of Polymers used, Evaluation of in situ gel and its applications.

KEYWORDS: In situ gel, Sol to gel, Polymer.

INTRODUCTION:

Over the last 30 years'the development of regulated and long-lasting medication delivery methods has received more attention. The design of polymeric drug delivery systems has been the subject of substantial investigation. The development of in situ gel systems has received a lot of attention in recent years[1]. In the last several years, a growing number of in situ gel forming systems have been studied, and numerous patents for their use in a variety of biological applications, including drug administration, have been published. In situ gel formulations offer an intriguing alternative to establishing systemic therapeutic effects through parenteral methods, which can result in incredibly low solubility and transit by hepatic first-pass metabolism, particularly for proteins and peptides. Because of its unique 'Sol to Gel' transition, the in-situgelling technology aids in the continuous and regulated release of medication, as well as increased patient compliance and comfort.

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Gastro retentive in situ gelling systems, also known as stomach-specific systems, have the capacity to give regulated medication delivery with improved gastro retention within the stomach. When in interaction with body fluids or a change in pH, in situ gelling systems are liquid at ambient temperature but gel when exposed to them[2]. Because the gel formed by the in-situ gelling system is brighter than gastric fluids, it floats above the contents of the stomach or adheres to the stomach mucosa because of bioadhesive nature of the polymer, resulting in dosage form retention and increased gastric residence time, resulting in prolonged drug delivery in the digestive tract[3,4]. A formulation that is in solution form before going the body, but changes to gel form below certain physiological conditions, is known as in situ gelling system. Temperature, pH change, solvent exchange, UV radiation, and the existence of certain molecules or ions all influence the sol to gel transition. Various natural and semi-synthetic polymers are gelled in situ and could be utilized for oral, ophthalmic, transdermal, buccal, intra peritoneal, parenteral, injectable, rectal, and vaginal administration. Pectin, gellan gum, chitosan, alginic acid, Carbopol, xyloglucan, xanthan gum, hydroxy propyl methyl cellulose, poloxamer, and other natural polymers are employed in the creation about in situ gelling systems[5-8].

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In situ gel system advantages[9,10]

- It aids in the administration of drugs.
- Its unique 'Sol Gel transition' aids in the regulated and prolonged release of the medication.
- The bioavailability of the drug will be higher.
- It aids in the reduction of drug administration frequency in the body.
- It can be administered to unconscious and old patients.
- There will be increased residence time of the drug due to gel formation.

In situ gel systemdisadvantages[11,12]

- The drug's sol form is more sensitive to deterioration.
- Only minimum dose can be given.
- It needs a large number of fluids.
- After drug administration, eating and drinking limited for a few hours.

Ideal Properties of polymers[13]

- It must be compatible and non-toxic.
- It should act in a Pseudoplastic manner.
- It should influence the tear behavior.
- The polymer must be able to stick to the mucosal membrane.
- The polymer should be able to reduce viscosity by increasing shear rate.

Fig No.1: ***Insitu*** **Gel**

In Situ Gel Mechanism:

Physical and chemical mechanisms are used to generate the in-situ gel system.

Physical Mechanism:

- **Diffusion:**

In situ gels are made using a type of physical process called diffusion. In this method, the polymer matrix is precipitated or solidified as the solvent from the polymer solution diffuses into the surrounding tissue. N-methyl pyrrolidone (NMP) is a polymer that is extensively utilized in the development of in situ gelling systems[14].

- **Swelling:**

In situ formulation uses a type of physical method called swelling. In this procedure, the polymer capsule is surrounded by fluids from the outside environment, which inflate from the outside to the inside, progressively releasing the medication. When glycerol (glycerol monooleate) is exposed to water, it expands and forms Lyotropic liquid crystalline phase structures. This material is bio adhesive and can be degraded in vivo by enzymes.

Chemical Mechanism[15]:

- **Ionic cross-linking:**

The ion sensitive polymer is used in this approach. Ion sensitive polymers may undergo phase transition in the presence of various ions such as Na+, K+, Ca+, and Mg+. Ion-sensitive polysaccharides are a type of polysaccharide. In the presence of a little amount of K+, k-carrageenan forms hard, brittle gels, whereas in the presence of Ca2+, I-carrageenan forms elastic gels. Gellan gum is commonly referred to as Gelrite. It's an anionic polysaccharide that gels in place when monovalent and divalent cations are present[16,17].

- **Enzymatic cross-linking:**

Enzymatic cross linking is the most appropriate method used in formation of in situ gelling system. In this process, gel is created by cross linking with the enzymes which are present in body fluids. In situ formation induce by natural enzymes and that are not been investigated widely but appears to have certain advantages over chemical and photochemical approaches. An enzymatic method, for example, can handle efficacy in physiological conditions without the use of potentially harmful chemicals like monomers and initiators. Hydrogels have been studied for application in intelligent stimuli-responsive insulin delivery devices. Modify the enzyme amount while maintaining an appropriate mechanism for managing the gel formation rate, which admits the mixes to be injected before gel formation.

- **Photo-polymerization:**

During the development of a system for in-situ gelling, electromagnetic radiations are utilized in the photo-polymerization procedure. A solution containing reactive macromeres or monomers, as well as invader, can be injected into a tissue location, and the gel can then be formed using electromagnetic radiation. The most ideal polymers for photo polymerization are those that dissociate by polymerisable functional groups in the appearance of a photo initiator such as acrylate or similar monomers and macromers, which are commonly utilized at long wavelength UV and visible wavelengths. Short wavelength UV is rarely employed since it penetrates tissue poorly and is biologically harmful. The initiator for UV photo-polymerization in this process is a ketone, like 2,2 dimethoxy-2-phenyl acetophenone. Camphorquinone and ethyl eosin initiators are used in visible light systems[18].

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Approaches of *In Situ* Gelation:

- **Temperature dependent in situ gel:**

In insitu gelling formulation, in ecologically sensitive polymer systems, temperature is the most commonly used stimulus. Both in vitro and in vivo, temperature changes are easy to manage and administer. Body warmth causes gelation in this technique therefore no external heat is required. These hydrogels are liquid at ambient temperature (20–25°C), but gel when they come into touch with body fluids (35–37°C). There are three types of temperature-induced systems. They are negatively thermos sensitive type example: Poly (Nisopropylacrylamide) Polyacrylic acid is a positively thermosensitive type; poloxamer, pluronics, and Tetronics are thermally reversible types. Thermo responsive or temperature responsive polymers are used in this system because they demonstrate a dramatic and discontinuous change in their physical characteristics as a function of temperature. These polymers exhibit a miscibility gap at high and low temperatures, indicating the presence of an upper and bottom essential solution temperature.

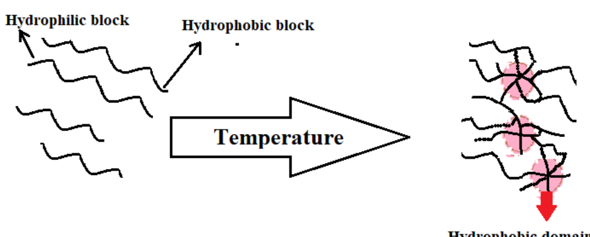

Fig no 2: Mechanism of Temperature dependent in situ gel system

- **pH dependent in situ gelation:**

In this system gel is formed due to pH changes. In this method pH sensitive polymers or pH responsive are used. pH sensitive polymers feature acidic or basic groups on their surface that can receive and released protons in accordance to changes in the overall pH[19]. Poly electrolytes are massive polymers with ionizable groups. The presence of poly electrolytes in the formulation produces an increase in external pH, causing the hydrogel to enlarge and form an in-situ gel. Polymers with anionic groups are suited for this method.CAP (cellulose acetate phthalate), carbomer and its derivatives, PEG (polyethylene glycol), pseudo latexes, and PMC (poly methacrylic acid) are a few examples.

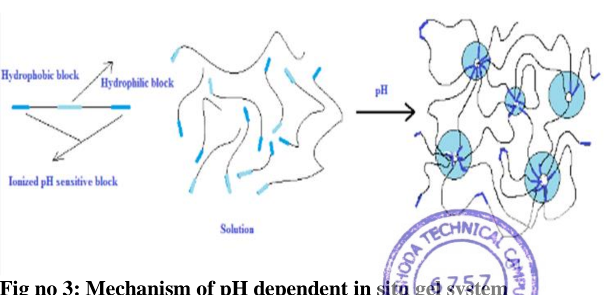

Fig no 3: Mechanism of pH dependent in situ gel system

- **Ion activated in situ gelation**

In this method, gelling of the solution instilled is triggered by change in the ionic strength. The amount of gelation is thought to be influenced by the osmotic gradient across the gel's surface. Gelrite or Gellan gum, Hyaluronic acid, and Alginates are examples of polymers that exhibit osmotically induced gelation[20,21].

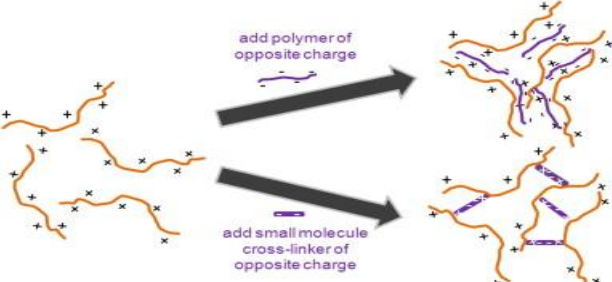

Fig no 4: Mechanism of ion activated in situ gel system

Polymeric System of *In Situ* Gel Classification:

- **Natural polymers:**
  Example: Pectin, Chitosan, Alginic acid, Gellan gum, Xanthan gum, Gaur gum, sodium hyaluronate, Carbopoletc.
- **Synthetic or semi-synthetic polymers:**
  Example: Hydroxypropyl methylcellulose, Cellulose acetate phthalate, methylcellulose, Poloxamer, Polyacrylic acid etc.

Natural Polymers:

- **Pectin:**

Pectinis a type of polysaccharide in which the majority of the polymer is make up of α-(1-4)-D galacturonic acid residues. In the egg-box model, less methoxy pectin (degree of esterification 50%) produce gels quickly in aqueous solution when free calcium ions interlink the galacturonic acid chains. The gelation of pectin, a source of monovalent, divalent, and trivalent ions, chance in the existence of H+ ions. Pectin can be used in these formulations without using organic solvents because it is water soluble. Divalent cations in the stomach aid in the transformation of pectin to a gel state when taken orally. Pectin is divided into two types:a) Low methoxy pectin:below than 50% of the carboxyl groups methylate the pectin. b) High methoxy pectin: greater than 50% of the carboxyl groups methylate the pectin[22].

- **Chitosan:**

Biodegradable, biocompatible, thermosensitive, pH dependent, cationic amino polysaccharide is produced by alkaline deacetylation of chitin. pH and temperature fluctuations cause chitosan to gel. It has good mucoadhesive properties because of the electrostatic interaction between cationic chitosan and anionic mucosal surfaces. Because of their availability, nontoxicity, and low cost, displaying polymers are employed to gel chitosan at higher critical solution temperatures[23,24].

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- **Sodium Alginate:**

Alginic acid is a linear block copolymer polysaccharide made up of 1,4-glycosidic links connecting β-D-mannuronic acid and α-L-glucuronic acid residues. The percentage of each block and the order in which the blocks are arranged along the molecule differ depending on the algal source. When divalent or trivalent metal ions are added to dilute aqueous alginates solutions, a cooperative mechanism involving sequential glucuronic remains in the α-L-glucuronic acid blocks of the alginate chain form solid gels. Alginic acid formulations were investigated for a longer precorneal stay, not only due to its ability to gel in the eye, but also due to its mucoadhesive properties[25,26].

- **Carbopol:**

Carbopol is a well-known pH-dependent polymer that remains in solution at acidic pH but gels at alkaline pH with a low viscosity. HPMC is used in conjunction with Carbopol to give the Carbopol solution viscosity while also lowering the acidity. pH-induced in-situ precipitating polymeric systems include a variety of water-soluble polymers such as the Carbopol system-hydroxypropyl methylcellulose system and poly (methacrylic acid)-poly (ethylene glycol). conceived and developed a pH-induced in-situ precipitating polymeric system (an aqueous solution of Carbopol-HPMC system) for plasmid DNA delivery[27].

- **Gellan gum:**

Gellan gum is an anionic hetero polysaccharide, secreted by microbe Sphingomonas elodea. It is produced from glucose, rhamnose, and glucuronic acid, which are joined to form a tetra saccharide molecule. Gelrite is deacetylated Gellan gum that has had the acetyl group in the molecule removed by alkali treatment. Gellan gum is used as a suspending and stabilizing agent in the food business[28].

- **Xanthan gum:**

Xanthan gum is a high molecular weight extracellular polymer generated by the gram-negative bacterium Xanthomonas campestis during fermentation. A cellulosic backbone (β-D-glucose residues) and a trisaccharide side chain of β-D-mannose-β-D-glucuronic acid α-D-mannose connected to alternating glucose residues of the main chain make up the major structure of this naturally generated cellulose derivative. Xanthan gum is dissolvable in both hot and cold fluid and is stable in acidic and alkaline environments. It is anionic because it contains both glucuronic and pyruvic acid groups[29,30].

- **Sodium hyaluronate:**

It is a water-soluble form of the sodium salt of hyaluronic acid. It's a natural, endogenous carbohydrate that helps the body produce collagen and keep its flexibility. It also increases formulation stability and reduces the portability of oxidation[31,32].

Synthetic or Semi- Synthetic Polymer:

- **HydroxypropylMethylCellulose (HPMC):**

The glucan chain in cellulose is prepared fromβ-(1, 4)-D-glucopyranose units that are repeated. Temperature sensitive sol-gel phase transition is observed in some natural polymers, such as HPMC, MC, and EC. When the temperature drops, cellulose material increases its viscosity, while its derivatives, such as HPMC and MC, increase their viscosity when the temperature rises. MC is a natural polymer made consisting of native cellulose chains with an alternative methyl substitution group. The solution is liquid at low temperatures (300˚C), but as the temperature increases (40-500˚C), it gels[33].

- **Cellulose acetate phthalate:**

Pseudo latex is cellulose acetate phthalate(CAP), which is a kind of cellulose acetate phthalate. It's a synthetic latex generated by dispersing a pre-existing polymer in water. Latex is a pH-sensitive, cross-linked polyacrylic polymer with potentially helpful qualities for sustained medicine distribution to the eye because it is a free-running solution with a pH of 4.4 that is raised to pH 7.4 by clotting tear fluid. The ocular duration of an ophthalmic preparation-scintigraphy is monitored using CAP, which does not require the use of an organic solvent[34].

- **Methylcellulose (Mc):**

Methylcellulose is a cellulose derivative that's employed as a gelling polymer in situ. At low temperatures, several cellulose derivatives remain liquid, but when heated, they turn into gels. The aqueous solutions of MC and HPMC, for example, at 40-50 °C and 75-90 °C, phase transition into gels respectively. The hydrophobic interactions between molecules containing methoxy groups cause HPMC and MC solutions to gel. Due to hydration at a lower temperature, macromolecules come into contact with each other. When the heat is increased, the hydration is gradually lost, resulting in a lesser viscosity[35,36,37].

- **Poloxamer:**

Poloxamer is a three-block copolymer that is water soluble. Poloxamer is sold as Pluronic and has a good thermal setting characteristic as well as a longer drug residence period. It's most commonly employed as a gelling, emulsifying, and solubilizing agent. Poloxamer produces a clear, colourless gel. Based on the ratio and distribution of hydrophilic and hydrophobic chains, many molecular weights are accessible, each with a distinct gelling behavior[38].

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- **Polyacrylic acid (PAA):**

PAA is commercially known to be Carbopol.It is commonly used in ophthalmology for increasing pre-corneal retention. It can exhibit excellent mucoadhesive properties to compare with another cellulose derivative[39].

Evaluation of *In* Situ Gelling System:

- **Clarity:**

Visual inspection against a black and white background can be used to check the clarity of prepared solutions.

- **Measurement of pH:**

The pH of each of the formulation was measured using a calibrated digital pH meter

- **Viscosity:**

The viscosities of the produced formulations were measured using a Brook field viscometer. it was sheared at 50 and 60 rpm using spindle number 63. The viscosity of each sample was measured three times.

- **Sol to gel time:**

Using a USP (Type II) dissolution equipment containing 500mL of 0.1N HCl (pH 1.2) at 370.50˚C. the in vitro gelation time was calculated. The gelling time is the amount of time it takes for an in-situ gelling system to gel for the first time. The gel floated on the buffer solution in a matter of seconds.

- ***In vitro* buoyancy study:**

The time it takes for the gel to rise to the top of the dissolution flask from the bottom is known as the floating lag time andthe floating period is the amount of time it takes for the generated gel to float on top of the dissolution liquid's surface is known as floating duration. In a USP type II dissolution test apparatus containing 500 ml of 0.1 N HCl (pH 1.2) at 370.50˚C.

- **Gel-Strength:**

The gel is made from the sol form in a beaker. This gel-filled beaker is elevated at a set rate, allowing a rheometer probe to gently pass through the gel. It can be determined by observing variations in probe load as a function of probe depth of immersion below the gel surface.

➢ ***In-vitro* drug release studies**

The plastic dialysis cell is used to conduct medication release experiments. The cell is made up of two half cells, a donor partition, and a receptor partition. The formulation's sol form is deposited in the donor compartment. In an incubator, the constructed cell is shaken horizontally. The entire volume of a receptor solution can be removed and replaced with new media at regular intervals. Analytical techniques are used to examine this receptor solution for drug release.

Application of *In situ* Polymeric Drug Delivery System:

- **Oral drug delivery:**

Natural polymers including pectin, xyloglucan, and gellan gum are employed to build oral medication delivery systems in situ. An in-situ gelling pectin preparation administered orally has been found to provide paracetamol for a long duration. The main advantage of using pectin in these formulations is that it is soluble in water, thus no organic solvents are required. According to the study, theophylline was administered orally using an in-situ gelling gellan formulation. The formulation included a gellan solution containing calcium chloride and sodium citrate complex. When calcium ions are given orally, they are discharged into the stomach's acidic environment, causing gellan to gel and create a gel in situ[40].

- **Ocular drug delivery system:**

Ocular delivery techniques frequently use natural polymers such as alginic acid, inulin, and xyloglucan. To release visual ocular tension in glaucoma, diverse chemicals such as autonomic medicines, anti-inflammatory agents, and antibacterial agents are employed in a local ophthalmic administration system. Because conventional administration systems generally result in poor availability and therapeutic response due to fast tear fluid turn over and dynamics, which leads to rapid drug removal from the eye, ocular in-situ gels were created to alleviate the bioavailability problem. Viscosity enhancers such as Carboxy Methyl Cellulose, HPMC, Carbomers, and Poly Vinyl Alcohol are used to increase viscosity in formulations to extend precorneal residence time and increase bioavailability while being simple to produce[41].

- **Nasal Drug Delivery Systems:**

In-situ gel was shown to reduce the increase in nasal symptoms when contrasted to the commercial formulation Nasonex (mometasone furoate suspension 0.05%). The presence of intact ciliated respiratory epithelium and usual goblet cell morphology in the rat nasal cavity indicated that these formulations were safe for nasal administration. Wu et al. Combining N- [(2-hydroxy-3methyl trimethyl ammonium) propyl] chitosan chloride and poly (ethylene glycol) with a small amount of – glycerol phosphate, researchers developed a novel thermos sensitive hydrogel for insulin delivery in the nose. At room temperature, the formulation was in solution form, but when stored at 37 °C, it converted into a gel form. As a result, these methods are suitable for the nasal administration of protein and peptide medicines[42].

- **Rectal and vaginal drug delivery system:**

Many types of medications can be delivered via the rectal route, including liquid, semisolid (ointments,

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creams, and foams), and solid dose forms (suppositories). Acetaminophen, an anti-inflammatory medicine, was formulated as a rectal in situ gel by employing polycarbophil, poloxamer F188, and poloxamer 407 as synthetic polymers generating in situ gelling liquid suppository, which is regarded to be an excellent way for increasing bioavailability. To improve therapeutic effects and patient compliance, a mucoadhesive, thermosensitive, prolonged release vaginal gel containing the clotrimazole-cyclodextrin complex was developed[43,44].

- **Injectable drug delivery system:**

Injectable in situ gel is mostly made up of synthetic polymers and block copolymers. A novel injectable thermosensitive in situ gelling hydrogel has been created for tumor treatment. The drug-loaded chitosan solution was neutralized with glycerol phosphate in this hydrogel. EMT-6 tumor implanted subcutaneously on albino mice were used to examine local delivery ofpaclitaxel from the intratumoral injected formulation. Ito et al. One example of inflammatory drug is Bupivacaine which is formulated as injectable in situ gel using poly(D,L-lactide), poly (D,L-lactide coglycolide) and PLGA as polymer shows prolong action drug in gel conditions[45,46].

CONCLUSION:

According to the current study, the 'in situ gel' system has appeared as one of the most effective drug delivery systems. The in-situ gel preparation was developed to improve patient compliance, comfort and lowering dose frequency. This approach increased residence and continuous release. It worked for both systemic and localization at the site of action. A variety of physiological parameterslike pH, temperature and ionic state which influence the gel's growth. In situ gel formation is used to generate a variety of natural, synthetic, and semi-synthetic polymers that could be utilized for oral, ophthalmic, nasal, rectal and vaginal and injectable drug delivery system.

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The Monkeypox Virus, methods to prevent the re-emergence of the Virus

Vinay Gaikwad[1*], Rajendra Kothalikar[1], Prakash Jadhav[2], Pankaj Khuspe[3], Swapnil Phade[3]
[1]Assistant Professor, Shikshan Prasarak Mandal's College of Pharmacy (Poly), Akluj, Maharashtra, India
[2]Associate Professor, Yashoda Technical Campus, Satara, Maharashtra, India
[3]Assistant Professor, Shriram Shikshan Sanstha, College of Pharmacy, Paniv, Maharashtra, India

[*]Corresponding Author: vinaygaikwadcopa@gmail.com

ABSTRACT

In Central and West Africa, the monkey-pox is a new and re-emerging zoonosis that occasionally results in fatal illnesses. The etiological agent of the complaint is monkey-pox contagion, a virus belonging to the family of orthopoxviruses. The State Serum Institute in Copenhagen initially identified monkey-pox infection in laboratory monkeys in 1958, and the Democratic Republic of the Congo reported the first fatal case of the disease in 1970. The primary spreaders of monkey-pox are African rodents.

The most frequent routes of infection for mortal beings are respiratory, percutaneous, and permucosal exposures to infected monkeys, zoo animals, champaign kids, and people. The incubation phase of the complaint lasts between 5 and 21 days, however it often lasts between 6 and 13. Most instances begin with a classic prodromal sickness that lasts for two days and includes fever, malaise, and lymphadenopathy. The face, triumphs of the hands, and soles of the bases are significantly affected by the rashes. The majority of instances are seen in people who have had direct contact with animals. However, monkey-pox can be predicted if the recognizable skin lesions are present and there is a history of exposure. Culture in the lab and polymerase chain reaction (PCR), Electron microscopy and immunohistochemistry are the instruments available for corroboration of the claim. In immunocompromised patients, the prognosis of the complaint is dismal. Care should be made to treat and cover fractures in the skin as a normal preventative measure while working with non-human primates or other mammals. Infection control measures, including as good hygiene, frequent hand washing, disinfection of shells and clothing, and the use of specific protective clothing (PPE), are crucial during trade with monkey-pox-affected animals.

Keywords- Animal mortality, Emerging, Monkey pox, Prevention, Zoonotic disease

INTRODUCTION

Emerging and re-emerging zoo-noses with various etiologies are important contributors to morbidity and death in both people and animals [1-3]. Several viral zoo-noses have attracted public health authorities' attention [1-6]. There are a number of zoonotic infections, such as cowpox, buffalo-pox, goat-pox, monkey-pox, and camel-pox that can infect both animals and people in different parts of the world [1]. In non-human primates, the pox-viruses cause four diseases, with monkey-pox being the most prevalent [7]. A sylvatic zoonosis called monkey-pox is infrequent and fatal in Central and West African wooded regions [8].

An important zoonotic disease that affects public health is monkey-pox [9, 10]. A smallpox vaccination can prevent monkey-pox in people since it is linked to smallpox in humans [7]. When two spurts of a complaint like a spell swept through monkey exploratory colonies in 1958, the term "monkey-pox" was coined [11].

In the Democratic Republic of the Congo, fatal monkey-pox was first identified in a human being in 1970. Since then, pastoral regions in Western Africa and the Congo Basin, particularly the Democratic Republic of the Congo, have demonstrated the instances' maturity. A significant epidemic occurred in the Democratic Republic of the Congo in 1996–1997. In the spring of 2003, instances of monkey-pox were confirmed in the Midwest of

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the United States of America, marking the disease's first known occurrence outside of Africa [8].

Epithelial pustule and vesicular lesions are brought on by the infection in both New and Old World monkeys and hams. Both the owner and the animal must have vaccinations in order to be protected [7]. The potential for monkey-pox to transmit from person to person and be exploited in bio-terrorism exists [10]. This mini-main review's goal is to outline the monkey-pox outbreak's emerging position as a zoonotic disease with public health implications.

PATHOGENESIS

The MPXV, an orthopoxviruses belonging to the family of monkey-pox viruses, is the culprit behind the disease [11, 12]. Other orthopoxviruses that cause diseases in humans include camel-pox, cowpox, vaccinia (used in the smallpox vaccine), and variola (smallpox). According to genome sequencing, there are two monkey-pox clades: Congo Basin and West African, and variations in lethal pathogenicity and mortality have been demonstrated in the two geographical locations [8]. The infection is an envelope, double-stranded DNA infection with a slightly pleomorphic core and side bodies, as well as the swine flu, West Nile fever, contagious ecthyma, Ebola hemorrhagic fever, Hantavirus infection, Rift Valley fever, Hendra hemorrhagic fever, Nipah hemorrhagic fever, and raspberry flu, have emerged from various world regions and drawn attention with a size of 140–260 nm in the perimeter and 220–450 nm in length [13]. It is resistant to phenolic detergents, but polar lipophilic detergents like chloroform and low pH render it inactive. The similarly related vaccinia contagion completely inactivates at 60°C in 2-3 hours, or twinkles at 22°C following exposure to 20nM caprylate [14, 15].

TRANSMISSION

Humans can get monkey-pox by being bitten by an infected animal or by coming into direct touch with the animal's lesions, blood, or bodily fluids [1, 4, 8, 11, 12]. Even if the complaint is less communicable than smallpox, it still has the potential to spread. The disease is thought to be spread by respiratory driblets during prolonged, direct face-to-face contact. Direct contact with an infected person's bodily fluids or contagious items, such as bedding or clothing, can also spread monkey-pox [11]. Another method of complaint transmission is vaccination or transmission via the placenta (natural monkey-pox) [8]. Direct contact with animals, especially rodents, is thought to be the most common way for complaints to spread [11].

EPIDEMIOLOGY

In 1958, monkey-pox infection was first connected to a spell infection in incarcerated monkeys [11]. However, the first case of fatal monkey-pox was reported in a 9-year-old kid from the Democratic Republic of the Congo in 1970. [8, 12]. Considering that fatal monkey-pox cases have also been recorded from a number of African nations, including Benin, Cameroon, Central African Republic, Cote d'Ivoire, Democratic Republic of the Congo, Gabon, Liberia, Nigeria, Sierra Leone, and South Sudan [8, 11]. In 2017, there was a severe human monkey-pox epidemic in Nigeria [8]. The 2003 epidemic of monkey-pox in the Midwest of the United States was the first known case of the disease outside of Africa. Prison pets like Champaign Tykes and other tiny animals, others who have come into contact with ill children from the Champaign region have estimated the maturity of cases around the country [8].

NATIVE HOST

A wide range of African life forms, including rope squirrels, tree squirrels, Gambian mice, banded mice, dormice, and primates, have been shown to carry the monkey-pox virus. Further research is necessary to ascertain the actual force of the infection and how it is sustained in nature. There are still unanswered issues regarding the contagion's natural history [8]. Funisciurus and Heliosciurus squirrels have received awards for their work as hosts and budgets [16]. According to American opinion, the disease was transmitted from several non-African animals (such champaign tykes) that the African creatures shared space with [8]. In laboratory experiments, the disease has been induced to infect mice, rats and rabbits [11].

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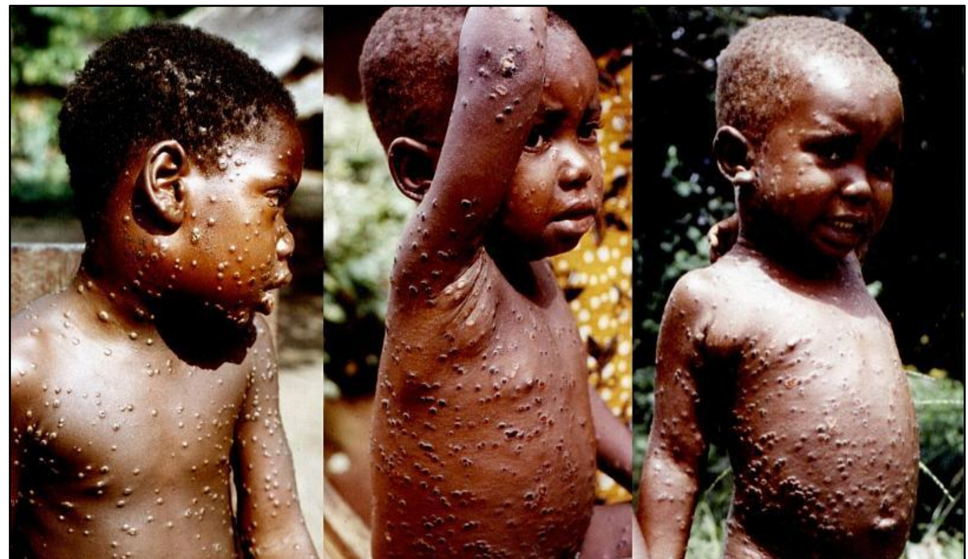
MEDICAL SYMPTOMS

The incubation period for monkey-pox is typically 7–14 days, although it may also be 5–21 days [8]. There are two phases to the illness. The irruption stage is marked by fever, intense headache, lymphadenitis, back pain, muscle pain, and extreme delicacy (lack of vitality) (0- 5 days), the time frame in which eruptions are occurring simultaneously on the face (in 95 of the instances), the palms of the hands and the soles of the feet (in 75 of the cases), and the torso. The progression of the rash includes the creation of crusts as well as macules, pustules, vesicles, and papules. Children who had monkey pox had rashes on several body parts (Fig. 1). [8]. Generalized exanthema and a severe systemic sickness with a homicidal expansion can develop in those who are immunocompromised or who have cellular susceptible response impairment. A hemorrhagic form of monkey-pox has never been transmitted to humans [17]. For fatal monkey-pox, the case-fatality rate is less than 10. Monkeypox-related losses mature in young cases, although [8]. In this setting, Jezzek and co-investigators [17] observed that 19 of the unvaccinated monkey-pox individuals had subsequent bacterial skin infections.



(Source: DRC, 1970-1977. -WHO /Mark V. Szczeniowski).

Figure 1: *A 7-year-old Zairian girl with monkey-pox in the acute stage, day 7 of rash, and monkey-pox in a 3-year-old Zairian boy with rash in the scabbing stage.*

A tone-restricting rash known as monkey-pox affects nonhuman monkeys. The earliest symptoms include fever and 1 to 4 mm dermatological pustules, which later turn into papules and crust over. A typical monkey-pox lesion has an epidermal hyperplasia ring around its red, septic, and depressed centre. Although these "papules" can be seen on the face, limbs, triumphs, soles, and tail, certain areas appear to have more of them [18]. Some species only have skin lesions. In severe cases, symptoms including coughing, sinus drainage, dyspnea, anorexia, facial edoema, mouth ulcers, or lymphadenitis maybe present. Circulated infection with deeper lesions is unusual in robotic infections. When exposed to an aerosol, monkeys only get pneumonia. Naturally infected creatures mature; nonetheless, losses can occur, particularly in stimulated monkeys. , infections might be asymptomatic [19].

OPINION

To create a clear opinion on monkey-pox, laboratory techniques such as contagion insulating, electron microscopy, immunohistochemical, and PCR are essential. The clinical manifestation of the complaint isn't really typical to make the opinion [4, 13]. Among the most prevalent discriminatory evaluations are those based on smallpox, chickenpox, measles, bacterial skin disorders, scabies, cure disinclinations, syphilis, and varicella infection [8, 13]. In clinical, veterinary,

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and monkey-pox virus-infected cell cultures, RT-PCR is frequently employed to detect monkey-pox infection DNA [20]. Immunoreactivity is used to describe viral antigens, and the enzyme-linked immunosorbent assay (ELISA) is used to detect IgG and IgM antibodies [21, 22]. The Polymerase Chain Response (PCR) System is regarded as Dependence on Laboratory Opinion because to the Delicacy and Perceptivity [8].

TREATMENT

Beast investigation proved that antiviral therapy with associated with negative effects. The treatment of Variola virus-induced human smallpox illness in adults and children with tecovirimat (also known as TPOXX or ST-246) is permitted by the FDA. The FDA does not, however, approve of its usage for diseases caused by other orthopoxviruses, such as monkey-pox. Animal mortality can be reduced more effectively by antiviral drugs than by administering the smallpox vaccination as a preventative measure [8, 22]. The way a matter is handled as it matures is indicative. The CDC advises that all animals with suspected monkey-pox be put to death to prevent the disease from spreading, even though many animals recover on their own and antiretroviral medications have been found to be beneficial in experimental infections. Isolation of the patient, protection of the skin and mucous membranes, Nutritional assistance, symptom relief, monitoring, and treatment of problems, as well as re-hydration therapy such symptomatic treatments are useful in monkey-pox infection [11].

CONTROL AND PREVENTION

Several precautions may be done to avoid contracting the monkey-pox virus. Avoid handling any materials that have come into contact with a sick animal, such as a coverlet. Separate diseased cases from those who could get infected. After coming into contact with infected individuals or animals, thoroughly wash your hands [13]. In the case of a monkey-pox pandemic, the animals can be immunized using the disease itself as a prophylactic measure [18]. The American Food and Drug Administration have granted ACAM2000 a licence to be used for smallpox vaccination in those who have been shown to be at a high risk of contracting the illness. Under an Expanded Access Investigational New Drug application, it has been made accessible for the treatment of monkey-pox illness (EA-IND). For those aged 1 and older who have been shown to be at high risk for infection to avoid monkey-pox illness, the CDC suggests that immunization with ACAM2000 might be taken into consideration. The U.S. Food and Drug Administration has authorized JYNNEOS^{TM} (also known as Imvamune or Imvanex), a reduced live virus vaccine, for the prevention of monkey-pox (Fig. 2) [11].

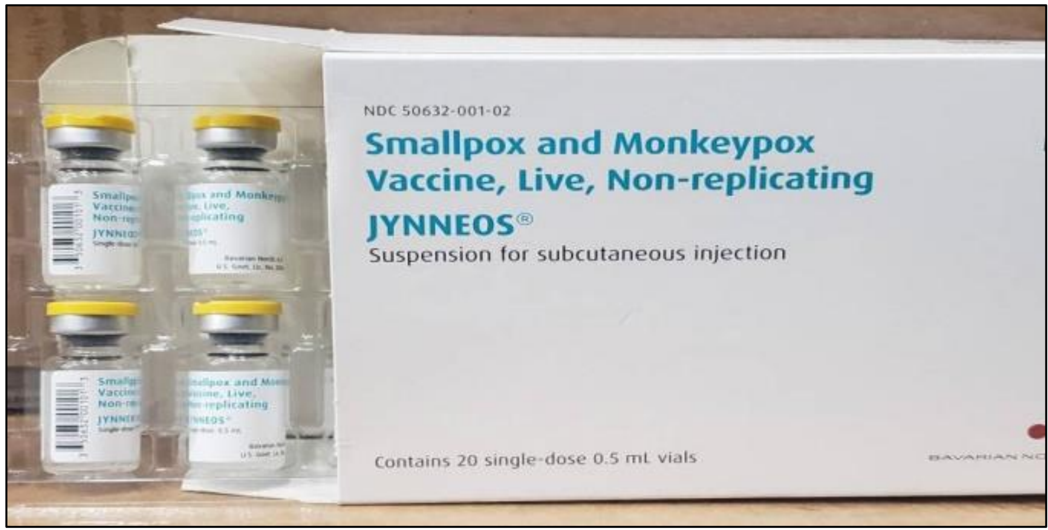

Figure 2: *Vaccines licensed by the U.S. Food and Drug Administration (FDA) are available for preventing monkey-pox infection - JYNNEOS (also known as Imvamune or Imvanex), Source: U.S. CDC 30 June 2022 [23].*

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As with restricting or banning the movement of small African animals and monkeys, preventing the transmission of monkey-pox through the pet trade has been effective in halting the disease's spread outside of Africa. Animals kept inside should not receive the smallpox vaccination. Instead, sick animals should be taken out of the herd and put in a counter blockade down low. Any animals that may have come into touch with the diseased animals should be quarantined for 30 days and checked for symptoms of monkey-pox. The only option to prevent infection in the absence of a specialized medication or vaccine is to increase public awareness of the risk factors and inform people of the steps they may take to reduce their exposure to the disease [8].

CONCLUSION

The monkey-pox virus is an emerging infectious illness that spreads from animals to people. The complaint frequently manifests clinically as a fever, rash, and blown lymph nodules. There may have been an immunological and ecological niche created for monkey-pox to reappear due to increased mortal-beast interaction as a result of climate change and deforestation, back country meat consumption, poor health, and inadequate exploratory structure, among other causes. Monkeypox is no longer just seen in native communities. As a result, monkey-pox infection is a risky, global resurgence of a disease. , there is no proven or secure method of treating a monkey-pox infection. The FDA has authorized JYNNEOS, a novel smallpox vaccine, to prevent both monkey-pox and smallpox. The need of practicing good hand hygiene is highlighted in order to prevent the transmission of illness after handling sick animals and contaminated natural objects. More research should be done on the severity, molecular epidemiology, and treatment of monkey-pox.

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REVIEW ARTICLE

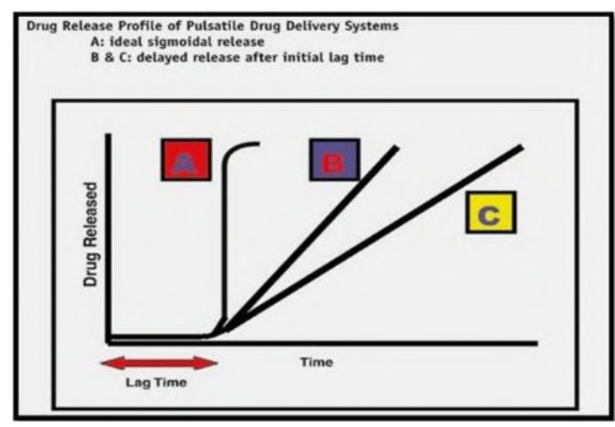
Pulsatile Delivery of Drug for a Range of Diseases

Sanket Nikam, Prakash Jadhav, Bharti Chaudhari, Atish Velhal
Yashoda Shikshan Prasarak Mandal, Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara.
*Corresponding Author E-mail: **sanketnikam222@gmail.com**

ABSTRACT:
Pulsatile delivery helps in providing the drug at an exact moment based on the disease's pathophysiology that cause improved patient therapeutic efficacy. Drug release rapidly after specific lag time this are advantage for many diseases condition. This system is designed according the body's circadian rhythm and after a lag time, the medication is released fast and totally as a pulse. Pulsatile delivery systems helpful in disease include asthma, arthritis, attention deficient syndrome, peptic ulcer and hypercholesterolemia. These systems are useful for diseases with chrono pharmacological behavior that necessitate nighttime administration, medications with a high first pass effect or GIT site specific absorption, and drugs with a high risk of toxicity or tolerance. By reducing dosing frequency, these devices help increase patient compliance. The foundation for this article is the disease that treat by pulsatile delivery system with drug used for specific disease condition and chrono pharmacology of all disease that mention in article.

KEYWORDS: Pulsatile delivery system, Lag time, Circadian rhythm, Chrono pharmacology.

INTRODUCTION:
In the new technologies the released of drug in immediate or extended fashion. Depending on the disease condition the development of drug release technology is modified.[1,2] PDDS is also part of that development in which it delivers the drug according to chrono pharmacotherapy of disease. This situation necessitates the release of the medicine after a period of time has passed. This situation may be produced using a pulsatile delivery system, which is described as the quick and transitory release of a certain amount of molecule in a short time period following a pre-set off release period (lag time). A pulse must be developed in this manner that the medicine is released completely and quickly. After the lag period, the medicine is released in sync with the body's circadian cycle.[3]



Were,
A] After a lag interval, a sigmoidal release occurs.
B] After a lag time, the release is delayed.
C] After a lag time, there is a sustained release.
Figure 1. Drug release profile of pulsatile drug delivery system.(2)

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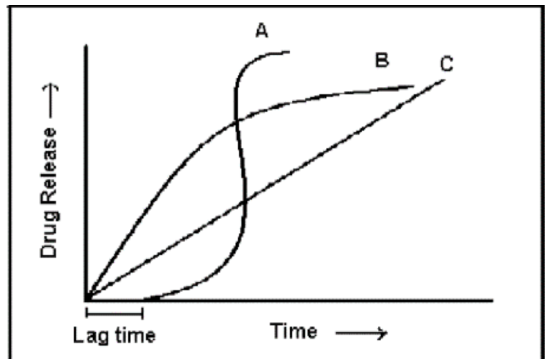

A] Pulsatile delivery system
B] Conventional drug delivery system
C] Extended drug release.

Figure 2. Drug release profile with compare to other drug delivery system and pulsatile delivery system (3)

In this graph 1Following the lag time, indicate sigmoidal release This is how pulsatile medication delivery systems will look in the future. When a medicine is released after a lag period, the drug is released all at once.[4]

Chrono Pharmacology:

It is a branch of research concerned with optimizing medicinal effectiveness and minimizing side effects by scheduling drugs in respect to biological rhythms.[5] The aim is to have a better knowledge of the periodic and thus predictable variations in medication's desired effects and tolerance. Chrono pharmacology is the branch of medicine that studies how drugs affect people's biological clocks.[6]

Rhythms in Human Physiology:

Rhythms in Human physiology are self-sustaining cycles that are innately determined by the length of time between subsequent repetitionsunder normal conditions. Within 24 hours, 100 different measured human body markers show cyclic variations.[6]

In medicine, circadian rhythms are particularly essential. Circadian rhythms (circa about dies, day, or about 24 hour) A physiological day lasts around 25 hours. The environment, night and day social schedules, reset the clock every day. [6] The brain's circadian clock regulates daily physiological cycles such as sleep/wake, digestion, temperature, and hormones. Endogenous circadian rhythms are biological rhythms. Free-running rhythms result from a lack of external synchronizers. The duration of free-running rhythms varies per species and can be longer or less than 24 hours. Our internal clocks are determined by our genes. In mammals, the suprachiasmatic nucleus of the hypothalamus (SCN) houses an internal biological clock that sends time signals throughout the body.[7] Circadian rhythms and annual/seasonal rhythms are controlled by it. To send out its time-of-day message, The SCN makes advantage of its autonomic nervous system connections, either by altering the endocrine glands' sensitivity or by directly influencing the endocrine output of the pineal gland (i.e., melatonin synthesis)

Rhythms of Different Systems:

- **Respiratory system:**

Greater bronchoconstriction at night due to increased parasympathetic tone, lower adrenaline and cortisol levels at midnight, and increased sensitivity to irritants and allergens at night.[7]

- **Gastrointestinal tract:**

Between 10 p.m. and 2 a.m., secretion of acid 2-3 times greater.[8]

- **Cardiovascular system:**

When comparing diastolic and systolic blood pressure, the amplitude of the 24-hour change is greater for diastolic blood pressure. At 9-11 a.m. and 6-7 p.m., blood pressure displays two peaks. Blood pressure drops (somewhat) in the afternoon and started decreasing at night.[9]

- **Endocrine system:**

Cortisol secretion is highest soon before awakening and lowest at mid-night in the morning. Growth hormone is at its highest during sleep. Early in the morning, testosterone peaks. 5-10 times increase in insulin Following the absorption of meals, the level rises.

- **Plasma protein binding:**

During nocturnal sleep, albumin and acid glycoprotein are at their lowest and highest levels, respectively. As a result, during night, medicines linked to plasma protein, such as lignocaine, carbamazepine, diazepam, valproic acid, and prednisolone, exhibit an increase in free fraction.

- **Liver enzymes:**

In the middle of the (nocturnal) activity span, oxidative reactions reach a high. UDP-glucuronyl transferees catalyze more conjugation during activity than at rest. During the rest period, sulphate conjugation is faster than during activity.

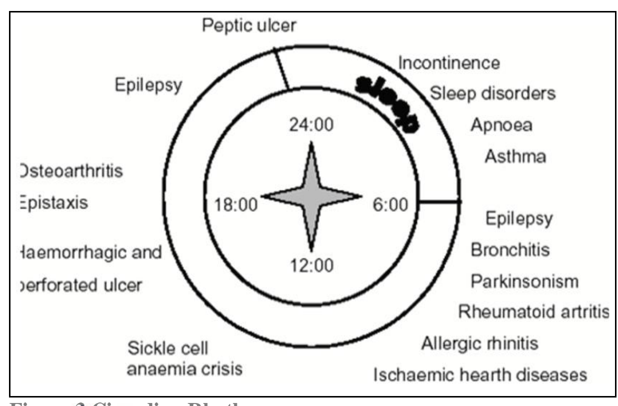

Figure 3 Circadian Rhythm

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Diseases and Chronotherapeutic:

24-hour cycle Up to now, the homeostatic hypothesis has guided the development of medication delivery methods. This notion is based on the idea that biological functions are consistent across time. Circadian rhythms have been identified for practically all human processes, including heart rate, blood pressure, body temperature, plasma concentrations of numerous hormones, stomach pH, and renal function, according to chronobiological research.[11] The importance of rhythmic processes in the therapy of human illnesses has become clear. Circadian rhythms exist in pathological stages of disease, just as they do in physiological processes. Epidemiological studies have shown that there is a higher chance of illness symptoms at this time.[12]

In the therapy of a variety of disorders, the potential benefits of chronotherapeutic have been proven. Patients with allergic rhinitis, rheumatoid arthritis and associated disorders, asthma, cancer, cardiovascular disease, and peptic ulcer disease are all interested in learning more about how chronotherapy can help them.[13] Patients with allergic rhinitis frequently report that their worst symptoms occur first thing in the morning. Patients taking a long-acting antihistamine at night instead of in the morning, as is typically suggested, may have better results in controlling morning pain.[14]

Diseases Required Pulsatile Delivery System:

1. Asthma
2. Peptic Ulcer
3. Cadiovascular Disease
4. Diebeties Mellitus
5. Hypercholestrolemia
6. Parkinson's Disease

Asthma:

Asthma is common chronic inflammatory disease of the airways characterized by hyperresponsive to variety of stimuli. Resistant airway in asthmatic patients, bronchoconstriction and symptom aggravation grow gradually during the night and early morning. Circadian alterations in lung function occur. For example, expiratory flow rates are the highest at 4 p.m. and lowest at 4 a.m. Approximately two-third of asthmatics suffer from nocturnal asthma symptoms and risk of asthma attack greater during night time sleep as compare to day time activity. This is driven by circadian changes in epinephrine (Bronchodilator), cortisol (ant inflammatory substance), Histamine (amedator if bronchoconstriction) melatonin (sleep regulatory hormone) AMP, vagal tone and body temperature. At 4.00 a.m., histamine concentrations peaked at a level that corresponded to the greatest degree of bronchoconstriction.[16,17] Pulsatile-release dosage form can potentially treat the nocturnal asthma by releasing drug after predetermine time delay, provided that most appropriate drugs are administrated. This dose type is given before bedtime, with medication release beginning in the early morning hours, when the risk of an asthmatic attack is highest.

Drug used in pulsatile delivery to treat nocturnal asthma:

Salbutamol:

Salbutamol is a fast-acting, highly selective Beta 2-adrenoceptor agonist with little cardiac adverse effects. This is used to treat asthma through relaxing the smooth muscle of the bronchial tubes causing the bronchi to dilate immediately.[16] The 2-4 mg pill is radially absorbed from the oral route, with a 44 percent absolute bioavailability and a peak plasma concentration of 1-3 hours.

Salbutamol has a short biological half-life (3.8 to 6 hours), a high first-pass metabolism, and a restricted therapeutic window when taken orally. A pulsatile delivery device exposes salbutamol sulphate only when it is needed, potentially preventing undesirable systemic side effects and allowing a lower dose of life-saving medication to be used to treat night-time asthma.[16]

Montelukast:

Montelukast is a drug that can be used to treat asthma and allergic rhinitis. Montelukast belongs to the class of drugs known as leukotriene receptor antagonists. It acts by inhibiting the function of leukotriene D4 in the lungs, resulting in less inflammation and smooth muscle relaxation.[17]

Theophylline:

Theophylline is a methylated xanthine class of medication used to treat respiratory illnesses such as COPD and asthma. It's a bronchodilator and nonselective phosphodiesterase inhibitor that improves respiratory muscle performance and mucociliary clearance. Theophylline's broncho dilating effect makes it effective in the long-term treatment of bronchial asthma.[18]

Peptic Ulcer:

Peptic ulcer is the erosion in lumen of stomach. The human stomach is capable of secreting hydrochloric acid in concentration that create a greater than 2-million-fold gradient in hydrogen ion concentration between the gastric lumen and tissue vascular compartment. Under the fasting condition acid is secreted in relatively low amount to maintain an intragastric pH of approximately 1.5. This slow level rate termed as basal acid secretion. The rate of basal acid secretion is highest between 9pm and midnight.[19]

Meals are often accompanied with an increase in acid secretion and a brief rise in intragastric pH because to the meal's buffering impact. Thus, during a day time. Intragastric pH changes over the day, particularly during

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mealtimes. Intragastric pH remains low during the night-time hours when there is no food present. Based on the Circadian rhythm, the secretion of gastric acid is higher in the early morning or late at night, so our goal is to give the medicine late at night and only then receive the right outcome. This condition is well treated by pulsatile delivery that provide a drug after required lag time.[19]

Drugs used in pulsatile delivery to treat peptic ulcer

Lansoprazole:

Lansoprazole is an antiulcer medication that belongs to the proton pump inhibitor class. Lansoprazole is a benzimidazole sulfoxide derivative that inhibits gastric acid output for a long time. Lansoprazole is used for the treatment of duodenal or gastric ulcers, gastroesophageal reflux disease, and zollinger-ellison syndrome. Lansoprazole, which can be targeted to the colon in a pH and time dependent manner, can be used to control medication levels in synchrony with nocturnal acid secretion's circadian rhythm.[20] A pulsatile 'Tablet in Capsule' dosage form containing lansoprazole taken at bedtime with a programmed start of drug release early in the morning hours can prevent a sharp increase in the incidence of high gastric acid secretion, especially during the early morning hours, when the risk of peptic ulcer is highest.

Rabeprazole:

Rabeprazole is medication that decrease stomach acid. It used to treat peptic ulcer, gastroesophageal reflux disease and excess stomach acid secretion. The effectiveness is similar to other proton pump inhibitors. Rabeprazole's bioavailability is approximately 52%, meaning that 52% of orally administered dose is expected to enter systemic circulation (the bloodstream). Rabeprazole is 97 percent protein bound once it reaches the bloodstream. Rabeprazole has a biological half-life of around one hour in humans. After a single orally given dosage, rabeprazole reaches its maximal level in human plasma in around 3.5 hours. Oral absorption is unaffected by the dosage given.[21]

Cardiovascular Disease:

Cardiovascular functions such as heart rate and blood pressure show 24 h variation. Since the majority of these illnesses can result in fatal or severe results, the incidence of cardiovascular disease such as acute myocardial infarction, strokes, and arrhythmia shows obvious diurnal variation. Cardiovascular medicine chrono pharmacology and chronotherapy: Implications for coronary heart disease prevention and treatment[22]

Various Cardiovascular Disease:

1) Blood pressure/Hypertension:

Blood Pressure is well known to exhibit 24 h variation with a peak in the morning. Throughout the day and night, blood pressure varies. The oscillations might last anywhere from seconds to minutes, or they can last a long time from day to night and season to season. The diurnal shifts associated with the sleep-wake cycle are the most easily noticed and important blood pressure variations[22] The pattern of blood pressure measurements acquired from a typical circadian rhythm during the sleep-wake cycle. During awake hours, the pressure rises, then plateaus for several hours until reaching a peak early in the morning.

2) Acute myocardial infraction (AMI):

The AMI Occurs in mostly early morning. A number of physiological functions. Throughout the day and night, blood pressure varies. The oscillations might last anywhere from seconds to several minutes, or they can last a long time from day to night and season to season. The diurnal shifts associated with the sleep-wake cycle are the most easily noticed and important blood pressure variations. The pattern of blood pressure measurements acquired from a typical circadian rhythm during the sleep-wake cycle. Continuous blood pressure monitoring throughout the day and night showed a trend with minimum systolic and diastolic pressure values between midnight and 4 a.m. In during waking time, the pressure rises, then plateaus for several hours before reaching maximum levels early in the morning.

Drug used in pulsatile delivery to treat cardiovascular disease:

Lisinopril:

Lisinopril is a drug of the angiotensin-converting enzyme (ACE) inhibitor class that is primarily used in the treatment of hypertension, congestive heart failure, and heart attacks. It is also used in preventing the renal and retinal complications of diabetes. The drug has a half-life of 12 hrs. This drug belongs to BCS Class III, having good water solubility.[23] Lisinopril is slowly and incompletely absorbed after oral administration with a bioavailability of 25–30%. The objective of the present study was to improve gastric retention, so consequently, the bioavailability of the drug. The distribution is expected to happen in a burst, that is, all at once after a lag period. The goal of developing an acceptable formulation is to deliver the medicine at the proper time, which is early in the morning.

Propranolol:

Propranolol is a competitive antagonist at beta adrenoceptors that is nonselective. It has a high affinity for both beta-1 and beta-2 receptors, but has a lower affinity for the beta-3 subtype. Despite its quick absorption after oral treatment, propranolol has such a limited bioavailability due to considerable first-pass metabolism. Liver metabolism eliminates propranolol, which is strongly bound to plasma proteins.

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Metoprolol:

Metoprolol is β1 selective adrenergic receptor blocking agent used in the management of hypertension, angina pectoris, cardiac arrhythmias, myocardial infarction, heart failure, hyperthyroidism and in the prophylactic treatment of migraine.[25] Because the half-life of the medicine is relatively short (about 4-6 hours) and drug administration is necessary every 4-6 hours in the normal course of therapy, sustained release/control release formulations are warranted for prolonging activity and improving patient compliance.

Atenolol:

Atenolol is a β1-receptor antagonist that is widely used in the therapyof diverse CVD such as angina pectoris, cardiac arrhythmia, and systematic HTN. In atenolol, the stereogeniccenter resides at the N-N-dimethyl propoxy side chain, resulting in the existence of enantiomeric pair. S-Atenolol is the S-enantiomer of (±)-atenolol, the eutomer, which alone is responsible for the β-adrenoceptor blockingactivity. The S-enantiomer has been found to lack the reported side effect of a lowered heart rate sometimes encountered with the racemate.

Diabetes mellitus:

In the management of diabetes, the target is to maintain the patient in normoglycemia. Since the time of day, patient activities, and medication timing can all affect the risk of blood glucose peaks and troughs, chrono pharmacological elements are extremely important in the management of diabetes mellitus, Shift workers are known to have greater incidences of diabetes and obesity, poor glucose control, and cardiovascular disease and death. The 24-hour circadian clock is crucial for glucose tolerance.[26] A human study discovered that when eating and sleeping cycles are not in sync with the internal body clock, blood sugar homeostasis and, in particular, glucose tolerance is significantly affected.

Drug used in pulsatiledelivery to treat Diabetes mellitus.

Glipizide:

Glipizide is belonging to class of sulfonylureas. Oral sulfonylurea act through insulin release by inhibiting the K_{ATP}channel of the pancreatic B-cell. Glipizide is an oral rapid and short acting anti-diabetic drug. The biological half-life of Glipizide is 3.4 to 0.7 hrs. to avoid multi dosing of drug pulsatile delivery system helpful to produce therapeutical effect at required time period.[27]

Hypercholesterolemia:

Hypercholesterolemia is a presence of high level of cholesterol in blood. High cholesterol can limit blood flow, increasing the risk of a heart attack or stroke. Cholesterol synthesis is generally high at night time as compare to daylight due to the activity of rate limiting enzyme HMG COA is higher at night. Diurnal cholesterol synthesis can account for up to 30-40% of total daily cholesterol synthesis. To treat this condition of high cholesterol synthesis at night HMG COA refuse inhibitors are used. Studies with HMG COA reductase inhibitor suggest that evening dosing is more effective than morning dosing.[28]

Drug used in pulsatile delivery to treat Hypercholesterolemia

Fluvastatin:

Fluvastatin sodium is an antilipemic agent that competitively inhibits HMG-CoA reductase. It's part of a class of drugs known as statins, and it's used to lower cholesterol levels in the bloodstream and prevent heart attacks and strokes. Its short biological half-life is 3 hrs. and low bioavailability makes it an appropriate candidate for pulsatile delivery system. Fluvastatin pulsatiledelivery is characterized by proportioning medication concentration in the early morning hours when free cholesterol levels are higher. It may be given before night and is capable of releasing drug after a predetermined time delay.[29]

Pravastatin:

Pravastatin is belonging to class of medication know as statin. This are the cholesterol lowering agent used to preventing cardiovascular disease at high risk and treating abnormal lipid conditions. Pravastatin inhibits function of HMG-COA reductase. The half-life of drug 1-3 hrs. It's recommended to use only after other measures, such as diet, exercises and weight reduction have not improved cholesterol level. The use of pravastatin is generally weaker as compare to other statin but for a pulsatiledelivery system they have an ideal approach due to its pharmacokinetics and minimum side effect.[30]

Parkinson's disease:

The loss of voluntary muscle movement is known as akinesia. It's most commonly associated with Parkinson's disease as a symptom (PD). Morning akinesia in Parkinson's disease Dopamine neurotransmission control is frequently disrupted, which leads to movement problems. Parkinson's disease (PD) is a movement illness caused by progressive dopamine neuron loss and is related with faulty dopamine neurotransmission control. In order to replace the lost dopamine PD motor symptoms is currently treated with oral levodopa (L-DOPA, a precursor of dopamine), which must be emptied from the stomach and absorbed in the proximal small intestine. Levodopa is converted into dopamine in the brain, and stored in the neurons until needed by the body for movement. It remains the single most effective agent in the management of Parkinson's symptoms.[31]

Drug used in pulsatile delivery to treat morning akinesia:

Levodopa and dopa decarboxylase inhibitor:

Pharmaceutical composition that addresses short-

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comings of current formulations comprising levodopaand DOPA decarboxylase inhibitors; by providing a composition that enables timed pulsatile release of these compounds. Providing a delayed burst release of a DOPA decarboxylase inhibitor such as carbidopa and a delayed burst release of levodopa after a predetermined lag time, preferably separated in time whereby the DOPA decarboxylase inhibitor is released before levodopa, provides a means for the management of morning akinesia in patients with Parkinson's disease.[31]

With pulsatile delivery, the patient may improve the night time sleeping pattern and be efficiently relieved from a complete disabling state in the morning. Furthermore, such a composition can be taken together with existing marketed immediate and controlled release levodopa products, to provide a full day dose coverage for most patients with Parkinson's disease.

It is an aspect to provide a pulsatile release pharmaceutical composition comprising levodopa and a DOPA decarboxylase inhibitor, and a pulsatile release component providing for a predetermined lag time followed by a pulse release of said levodopa and said DOPA decarboxylase inhibitor.

CONCLUSION:

The development of a pulsatiledelivery system that can effectively treat diseases with non-constant dose therapy has made significant progress. Due to high patient compliance, convenience of administration, and versatility in formulations, oral medication delivery is currently the most preferred route of drug delivery. Hypertension, osteoarthritis, peptic ulcer, asthma, and other circadian disorders necessitate chrono pharmacotherapy. The methods described in this article are based on attempts made over the last decade to achieve Pulsatile release for a variety of diseases.

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REVIEW ARTICLE

A Review on Diverging approaches to Fabricate Polymeric Nanoparticles

Shivani Deshmukh, Bharati Chaudhari, Atish Velhal, Vivekkumar Redasani.
Yashoda Shikshan Prasarak Mandal, Yashoda Technical Campus,
Faculty of Pharmacy, Wadhe NH4, Satara. 415011.
*Corresponding Author E-mail: **shivadeshmukh26@gmail.com**

ABSTRACT:
Polymeric nanoparticles (NPs) are tiny particles with a diameter ranging from of 1 to 1000 nm that can be loaded with active compounds or surface-adsorbed onto the polymeric core. It is novel technology by which we improved therapeutic efficacy, bioavailability of API and better patient compliance. Polymeric NPs have shown promising in the delivery of drug to specific sites for the treatment of a range of disease. Polymeric nanoparticles with a size in the nanometer range protect active substance against degradation in vitro and in vivo, release the drug in a controlled manner, and allow for targeted therapy. The current review discusses polymeric nanoparticle manufacturing methods. Using polymeric drug nanoparticles to improve the therapeutic effectiveness of poorly soluble medicines in any mode of delivery is a universal approach.

KEYWORDS: Drug Delivery System, Polymer-Drug, Biodegradable polymers, Nanoparticles.

INTRODUCTION:
Polymeric nanoparticles (NPs) have sparked a lot of attention in recent years owing to its unique features that come from their small size[1-2]. Polymeric NPs as drug carriers have several features, including the capacity to control drug release, shield drug and other biologically active compounds from the environment, and improve bioavailability and therapeutic index[3-4]. The term "nanoparticle" refers to both nanocapsules and nanospheres, which are morphologically distinct[5-6]. Nanocapsules have an oily core in which the medicine is normally dissolved, and a polymeric shell that regulates the drug's release profile from the core[7]. The medication can be maintained inside or adsorbed onto the surface of nanospheres, which are made up of a continuous polymeric network[8-9]. The reservoir framework (nanocapsule) and matrix system (Nanosphere) are two different forms of polymeric NPs[10-11]. Among the most intriguing techniques to obtaining local controlled therapeutic delivery is the use of polymeric nanoparticles.

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Polymeric nanoparticles are colloidal solid particles with a diameter ranging of 10 to 1000nm that can be spherical, branched, or shell structures built from biodegradable and non-biodegradable polymers, wherein substances are embedded into nanoparticles through dissolution, entrapment, adsorption, and attachment, or encapsulation[12]. In order to ensure effectiveness, polymeric nanoparticles are a major advance over traditional oral and intravenous methods. Polymeric nanoparticles could be efficiently utilized in numerous drug delivery operations, such as tissue engineering, as well as therapeutic delivery for non-human animals[13]. Polymeric nanoparticles are attractive candidates for treating cancer, vaccine administration, contraception, and delivery of targeted antibiotics due to their choice of polymer and capacity to adjust drug release via polymeric nanoparticles[14-15].

Advantages of Polymeric Nanoparticle: [17]

- Controlled and sustained release of active during transit and at the point of administration, modifying the drug's organ distribution and subsequent clearance in order to improve therapeutic potential and reduce side effects.
- Lower toxicity and the occurrence of adverse medication responses, as well as improved drug

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consumption.

- Targeting ligands could be attached to the surface of the particles or magnetic guiding is used to accomplish site deliberate targeting.
- The system can be used in a diversity of treatment modalities, such as oral, nasal, parenteral, intra ocular, and so on.

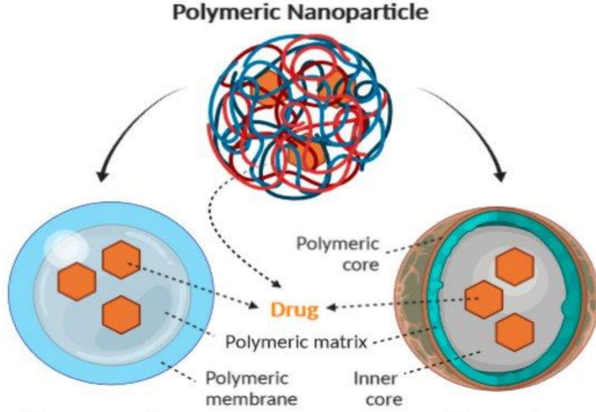

Fig 1: Diagram of Polymeric Nanoparticle[16]

Different Approaches for Synthesis of Polymeric Nanoparticle:

The technological advancement of emulsification equipment has prompted the development of the solvent evaporation technique, which has prompted the development of methods for emulsion preparation with nanoscale droplets over the last decade. Although this procedure is simple and adaptable, it can only be used with liposoluble medicines, it is time consuming, and nanoparticle coalescence during evaporation is a possibility[18].

Emulsification-solvent diffusion:

It involves creating a traditional o/w emulsion from a somewhat water-miscible solvent containing the polymer and medication and an aqueous solution containing a surfactant. To establish the initial thermodynamic equilibrium of both liquids, the polymer solvent and water must be mutually saturated at room temperature for this approach to work. The development of colloidal particles is induced by solvent diffusion from the dispersed droplets into the exterior phase after dilution with a large volume of water [19-20]. Diffusion rather than direct evaporation of the organic solvent from the nanodroplets is a more gentle procedure. Unlike approaches based on solvent evaporation, the droplet size in this technique falls abruptly over a millisecond time scale during solvent diffusion[21-22]. This process is usually used to make nanospheres, but it may also be used to make nanocapsules by simply adding a small amount of oil. Finally, evaporation or filtering can be used to remove the solvent, relying on its boiling point.

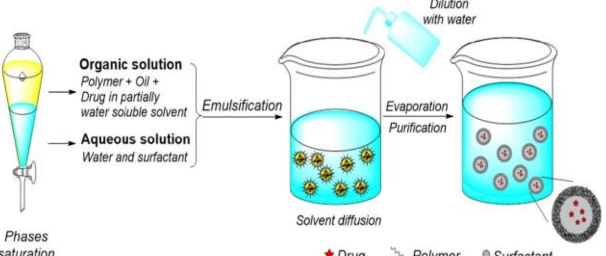

Fig 2: Diagram of Emulsification-solvent diffusion[23].

Emulsification–reverse salting-out:

The recently reported emulsification solvent diffusion approach can be thought of as a refinement of the emulsification-reverse salting-out method[24-25]. The key distinction is in the emulsion's composition, which is made up of a water-miscible polymer carrier such as acetone and an aqueous gel comprising the salting-out agent and a colloidal stabilizer. Electrolytes like magnesium chloride, calcium chloride, or magnesium acetate, as well as non-electrolytes like sucrose, are good salting-out agents. The emulsification is accomplished using the Ouzo effect rather than high-shear forces [26-27]. By saturating the aqueous phase, the miscibility of acetone and water is lowered, allowing the production of an o/w emulsion from the otherwise miscible phases. Dilution of the produced o/w emulsion including an excess of water promotes the diffusion of acetone into the aqueous medium, resulting in the precipitation of the polymer dissolved in the emulsified nanodroplets, resulting in a reverse salting-out action. Cross-flow filtration removes the leftover polymer solvent and salting-out agent[28-29]. Adequate miscibility of the organic solvent and water is not required, although it facilitates the execution process. If this is not the case, a higher water/solvent volume proportion will be required throughout nanoparticle production.

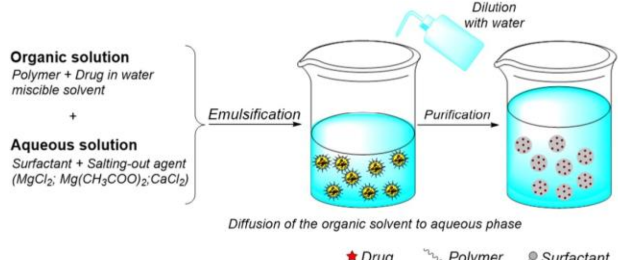

Fig 3: Diagram of Emulsification–reverse salting-out [23].

Nanoprecipitation method:

This technique's core premise is based on the interfacial deposition of a polymer when the organic solvent is displaced from a lipophilic solution to the aqueous environment. The polymer is dissolved in an intermediate polarity water-miscible solvent and then introduced to a stirred aqueous phase in a single shot, sequentially, dropwise, or by controlled addition rate[30]. Nanoparticles form instantly in an order to dodge water

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molecules due to the fast spontaneous diffusion of the polymer solution into the aqueous phase. The Marangoni effect appears to govern this phenomenon, in which a decrease in the interfacial conflict between two phases increases the surface area due to fast diffusion, resulting in the creation of tiny droplets of organic solvent[31]. The polymer precipitates in the form of nanocapsules or nanospheres as the solvent diffuses out of the nanodroplets. The organic phase is typically introduced to the aqueous phase; however the procedure might be reversed without impacting nanoparticle production. Acetone is the most commonly used organic solvent since it is miscible with water and quick to evaporate. Nonetheless, ethanol and binary solvent mixes, such as acetone with a tiny amount of water, ethanol, or methanol, can be utilised [32]. As long as the solubility, insolubility, and miscibility requirements are met, either two organic or two aqueous phases can be used. Surfactants are often used in the technique to enhance the integrity of the colloidal suspension, but their existence is not needed for nanoparticle creation. The resultant nanoparticles are typically well-defined in size and have a limited size variation, which is superior to that, obtained using the emulsification solvent evaporation technique [33].

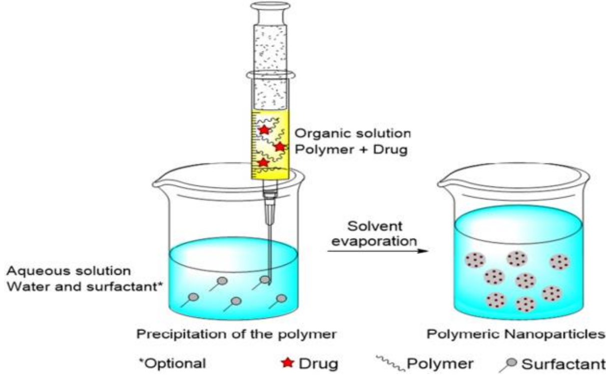

Fig 4: Diagram of Nanoprecipitation Method [23].

Dialysis:

The dialysis approach has been used to make small PNPs with a restricted size distribution successfully [34]. It's regulated by a method similar to that of the nanoprecipitation technique, but with a bit distinct test design. As a physical barrier for the polymer, dialysis tubes or semipermeable membranes with a sufficient molecular MWCO are employed[35]. The polymer is usually emulsified, inserted into the dialysis membrane, and dialyzed against a non-solvent. The inclusion of dilute polymer solutions and the miscibility of the solvents are also essential requirements. As the solvent within the membrane is displaced, the mixture becomes less capable of dissolving the polymer. Furthermore, a rise in interfacial tension causes. Furthermore, an elevation in interfacial tension causes polymer agglomeration and the creation of a colloidal nanoparticle suspension. Whereas dialysis is a straightforward and frequent approach, the enormous volume of counter dialyzing liquid could cause the nanoparticle payload to be released prematurely due to the lengthy process.

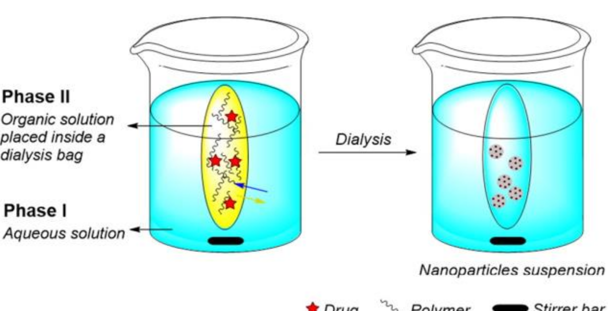

Fig 5: Diagram of Dialysis method[23].

CONCLUSION:

Nanomedicine holds the prospect of developing novel therapeutic platforms that are more effective and have fewer negative effects than traditional formulations. The advantage of delivery systems with nanoscale dimensions is that they have the highest volumeto-size ratio of any dose form. Nanoplatforms have been used in the field of oral medication administration to improve drug solubility, absorption, and bioavailability due to this feature. Nanoformulations also offer the potential to safeguard labile APIs and control drug release, as well as to treat chronic GI illnesses by site-specific and target-oriented delivery.In particular, the development of nanocarriers for oral drug administration has covered three primary sectors of application, in our opinion: increasing the bioavailability of APIs in BCS classes II and IV, treating specific GI regions locally, and delivering biotherapeutics (protein, peptide, and nucleic acids).

To summarise, nanomaterials research for oral medication delivery has recently seen a diversification of material kinds and a rise in the complexity of formulations, as well as the development of new "smart" nanosystems.

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REVIEW ARTICLE

Pharmacosome as a Vesicular Drug Delivery System

R. R. Shinde*, B. P. Chaudhari, A. B. Velhal, V. K. Redasani
Yashoda Shikshan Prasarak Mandal's, Yashoda Technical Campus,
Faculty of Pharmacy, Wadhe NH4, Satara. 415011.
*Corresponding Author E-mail: **shinderenuka30@gmail.com**

ABSTRACT:
In the area of solubility enhancement, several problems are encountered. Pharmacosomes are a new technology based on lipid drug carriers. Pharmacosomes are colloidal, vesicles, nanometric size micelles or a hexagonal arrangement of colloidal drug dispersions covalently attached to the phospholipid. Because of their unique properties such as active drug loading, small size, high entrapment efficiency, amphiphilicity and stability, they act as a precise drug carrier. They contribute to the controlled release of medicament at the action site, as well as the reduction of therapy cost, increased bioavailability of poorly soluble drugs, decrease toxicity and drug leakage. There has been an advancement in the scope of this delivery system for a number of drugs used for cancer, heart diseases, inflammation and protein delivery besides with a large number of herbal drugs. Pharmacosomes offer new opportunities and challenges for developing a more effective new vesicular drug delivery system.

KEYWORDS: Pharmacosomes, Solubility enhancement, Carrier, vesicular system, phospholipid.

INTRODUCTION:

BCSis a scientific categorization of a drug substance on the basis of its water solubility and intestinal permeability. After oral administration of a drug, it gets dissolved into the gastric (hydrophilic) fluid initially, and then permeated across the biological membranes (lipophilic) and finally reaches in the blood. Many synthetic and natural medications have limited absorption or penetration through the biological membrane, which limits their absorption and total availability to the body system[1].

Poor absorption may be due to their poor water solubility, whereas poor permeation may be due to the poor miscibility with the lipids, thereby severely limiting their capability to pass over the lipid-rich cell membranes of the small intestine.

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Therefore, a greater no. of strategies including soluble pro-drug, solid dispersions, cyclodextrin and phospholipids, and vesicular drug delivery systems, etc have been investigated to improve the absorption and penetration of biologically active molecules[2-4].

Vesicular drug delivery systems (VDDS) are important systems used for drug targeting, increasing the bioavailability and stability. These systems have an aqueous core normally adjoining by a lipid bilayer. The system is used as vehicle for the delivery of both water soluble and water insoluble types of drugs. The hydrophilic drugs are entrapped in the inner aqueous core, while hydrophobic drugs are encapsulated in a lipid bilayer. The various advantages offered by VDDS are tissue targeting, high drug entrapment, long retention time, reduced side effects, and increased bioavailability. Furthermore, the system can deliver a drug to the site of action at predetermined rate[5-7].

However, beneficial VDDS have certain limitations. The chief limitations of VDDS are linked to their preparation method, stability, scale-up, loading efficacy, cost-effectiveness, sterilization, burst release, and short half-

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life. Therefore, the pharmacosomes were developed to reduce various defect associated with the conventional vesicular delivery systems. The pharmacosomes are reported as lipophilic prodrug conjugates that self-assemble.

In this review, overview in a very comprehensive manner, the pharmacosomes as an important vesicular delivery system, different components and techniques of preparation and characterization of pharmacosomes, and their applications. In addition, we have analysed the pharmacosomes of different drugs prepared using variety of lipids and their effects on physicochemical properties and pharmacokinetic performance of the drugs. Finally, we conclude by outlining future outcome for the development of pharmacosomes drug delivery.

Pharmacosomes:

Pharmacosomes are colloidal dispersions where the active medicament is covalently bound to the lipid which gives rise to an amphiphilic block. Based on chemical arrangement of drug lipid complex, they reside as fine to an extreme degree of vesicular, micellar, and hexagonal aggregates. The development of vesicular pharmacosomes originates from the surface and bulk interlinking of drugs and lipids. The drug possessing the active functional groups (–COOH, -OH, -NH2) can be covalently linked to lipids with or without spacer chain by esterification or any other suitable conjugation strategy leads to formation of prodrugs. These prodrugs behave like amphiphilic molecules and get self-assembled in one or more layers in contact with the aq. medium. These layers further self-assembled inform of vesicles which resulting in formation of pharmacosomes. In pharmacosomes the drug molecules act as polar head and attached lipids as a non-polar tail. Pharmacosomes avoid problems such as drug leakage, drug incorporation, and reduced shelf life. They can improve bioavailability of drug due to the depletion of interfacial tension, increased area of contact. Pharmacosomes stability is depends on the physical and chemical characteristics of the conjugate system. It possesses several advantages above other vesicular systems such as transferosomes, niosomes, liposomes and hence serves as an alternative to this vesicular systems[8].

Pharmacosomes plays a vital role in the improvement of the drugs dissolution in gastrointestinal fluid and enhancement of their permeation through the lipophilic membrane. Besides, they can enhancedrugs bioavailabilityhaving either low lipid or/and water solubility. The prodrug approach has a high drug trapping efficacy and effectively avoids vesicle leakage and bursts release. As a result, the step of removing the unbound or unentrapped medication from the process of formulation can be skipped, which is a major restriction of liposomes. Pharmacosomes stability is mainly depends on the physiochemical properties of drug-phospholipid complex like solubility, melting point, phase transition temperature, and lipid composition[9-10]. The types of functional groups present in the drug molecule, the length of fatty acid chains in lipids, and the presence or lack of spacer groups all affect the rate of pharmacosome breakdown into active drug molecules. To achieve the desired in vivo pharmacokinetic behaviour, all of these parameters can be tweaked individually. It is reported that a lot of drugs including anti-cancer, cardiovascular drugs, non-steroidal anti-inflammatory drugs (NSAIDS), proteins, and herbal products are delivered through pharmacosomes. Pharmacosomes can be given through different routes like topical, oral and extra vascular routes[11,12].

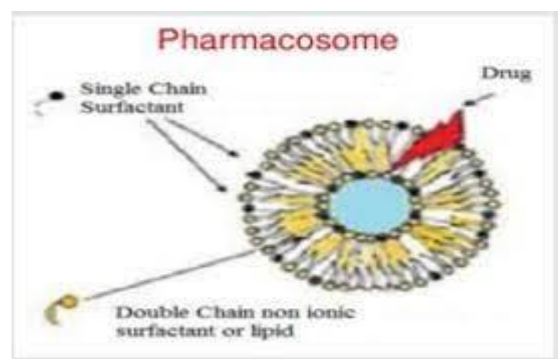

Fig. 1. Structure of pharmacosome

Advantages[13,14]:

1. Drug can be delivered to the active site of infection.
2. Improve bioavailability especially in the case of hydrophobic drug.
3. Reduction in adverse effect and toxicity.
4. Drug carriers like liposomes, nanoparticles, micro emulsions which lead to low physical stability and low drug-loading efficiencysuch as sedimentation, aggregation, and drug leakage during preparation, etc is not present in pharmacosomes.
5. Easily incorporate the drug.
6. Entrapment efficiency is high and predefined since the medication and carrier are covalently bonded and form a stoichiometrically specified unit.

Disadvantage[15]:

1. It requires surface and bulk interactivity of lipids with drugs.
2. To protect the leakage of drugs it requires covalent bonding

Application[16-18]:

1. Pharmacosomes show wider stability profile and longer shelf life.
2. Pharmacosomes has capacity to increase drug absorption and conveyance. Using response surface design, the formulated pharmacosomes were optimized and their attributes examined by

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colleagues.

3. Pharmacosomes can increase the permeation rate by improving the fluidity ofmembranes. The approaches have successfully improved therapy, performance, and various drugs such as pindolol diglyceride, amoxicillin, Taxol, cytarabine, dermatansulfate, bupranolol hydrochloride, and so on.
4. Pharmacosomes have a greater degree of selectivity for targeted drug delivery.
5. Pharmacosomes have reach a new level by amplifying therapeutic effects of many drugs like cytarabine, Taxol, dermatan sulphate, pindolol derivative, amoxicillin and so forth.

Component of Pharmacosomes:
The three main components of pharmacosomes are drug, lipid and solvents.

Drug:
A drug having active hydrogen atom (-OH, $-NH_2$, -COOH,) esterified with a lipid moiety to get amphiphilic block. This amphiphilic block facilitates drug transport via cell membrane, tissue and cell wall. Pharmacosomes of several drugs such as diclofenac, aceclofenac, geniposide, aspirin etc were prepared.

Solvent[19]:
Solvents with high polarity and solubility used for preparation of pharmacosomes. Highly purified and volatile solvent must be used. Generally solvents with intermediate polarity such as acetone, dichloromethane, ethanol, methanol, tetrahydrofuran etc. were preferred for preparation of pharmacosomes.

Lipid[20,21]:
The basic component of a biological membrane is phospholipids. Thephospholipids are of 2 types such as sphingolipidsandphosphoglycerates which are majorly used. Phosphatidylcholine is commonly used lipid for the pharmacosome preparation. It is an amphiphilic block in which hydrophobic acyl hydrocarbon chains pair binds with a water-soluble polar head group of phosphocholine with glycerol bridge. Phosphatidylcholine helps to maintain purity of cell membrane and involved in various biological processes. It acts as source of protein and hepatoprotective agent and utilize in the control of liver disorders. Besides, it prevents fibrosis and also the cirrhosis by enhancing collagen breakdown. Furthermore, it is used to treat different brain conditions such as memory loss, Alzheimer disease, and tardive dyskinesia, and in cancer management. The chemical structure of different lipids used for pharmacosomes preparation.

Table no. 1. Difference between pharmacosomes and liposomes[17]:

Liposomes		Pharmacosomes
Principle	Incorporation of drug in the aq. or lipid phase ofmixture of lipid where the physicochemical properties of the carrier and release of drug will be functions of different lipids used.	The covalent binding of a medication to a lipid results in a molecule that serves as both a carrier and an active agent. The physicochemical properties depend on drug as well as the lipid.
Loss of drug	Through leakage	No leakage, since drug is covalently bound but loss of drug by hydrolysis is possible.
Manufacturing	Cast fill method Extrusion/sonication Injectable method Reverse phase evaporation etc.	Self- dispersion through moderate mixing and sonication.
Separation of free drug	By gel filtration, dialysis, ultrafiltration, ultracentrifugation.	Not necessary since the drug covalently linked.
Volume of inclusion	Decisive in incorporation of drug molecules.	Irrelevant, since the drug is covalently bound
Surface charge	Achieved through lipid combination.	Depends on the physicochemical structure of complex.
Membrane fluidity	Depends on lipid combination and presence of cholesterol fluidity influences the rate of drug release and physical stability of system.	Depends on drug lipid complex phase transition temp. The drug is covalently bound, hence there is zero effect on release rate.
Release of drug	Diffusion through the bilayer, desorption from the surface or release through degradation of liposomes.	Hydrolysis (including enzymatic).
Physical stability	Relatively good Aggregation through double valanced cation.	Depends on physicochemical properties of drug and lipid complex.

Methods of Pharmacosomes Preparation[22-25]:
The pharmacosomes can be prepared by different methods. The methods of pharmacosomes preparation are discussed below.

1. Hand shaking method:
It is one of the simple methods of pharmacosomes preparation where the drug lipid mixture is dissolved in an organic solvent which is volatile in nature in a RBF. Then, the solvent is permit to evaporate utilizing a rotary vacuum evaporator that leads the formation of a thin film in a flask. Finally, the thin film is hydrated using an aqueous medium which offer a vesicular suspension.

2. Ether injection method:
Thecomplex of drug and lipid is dissolved in a definite quantity of ether. This ether solution is then injected in hot buffer or aqueous medium, where vesicles get formed. Vesicles may be in different forms such as round, cylindrical, cubic, or hexagonal type. The shape of vesicles depends on the amphiphilic nature of compound and its concentration.

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3. Anhydrous co-solvent lyophilization method:

The drug and phospholipid are dissolved in solution of DMSO and glacial acetic acid. Then, this mixture is blend to form a clear liquid solution and freeze-dried at whole night at condenser temperature. The complex obtained is flushed with nitrogen. Then stored at 4^0C.

4. Solvent evaporation method:

It is a conventional method of pharmacosomes preparation where the drug is firstly acidified to get reactive hydrogen atom which is necessary for complexation. An acid solution of drug extracted with chloroform then recrystallized. The drug lipid complex is dissolved in the organic solvents in an RBF at different ratios. The resultant mixture then refluxed for 1 or 2 hours. Then dried under vacuum evaporator at 40^0C. This dried residue placed in a vacuum desiccator for complete drying. It is time-consuming and involves multistage processing.

5. Supercritical fluid process:

This method is used to overcome shortcomings related with the solvent evaporation technique. The main drawbacks of solvent evaporation technique, time-consuming and involve multistage processing. Besides, the dissolution of pharmacosomes does not improve ideally. Parameters allied to solid morphology, including thecrystal pattern, crystal habit, and particle size, affect the dissolution rate of a compound hence affect bioavailability. The two main techniques used in the supercritical process are gas anti-solvent and solution improve dispersion by supercritical fluid. In this method drug lipid complex is dissolved into supercritical fluid of CO_2 and mixed by using a nozzle mixing chamber. Pharmacosomes formed by fast mixing of dispersion due to the turbulent flow of carbon dioxideandsolvent.

Characterization of Pharmacosomes:

Characterization of drug-lipid complex (prodrug):

1. Chromatography[26]:

The simple chromatographic technique like TLC is primarily used for the confirmation of prodrug. The purity of starting materials and product as well as the progress of drug-phospholipid conjugate synthesis is confirmed by this technique. Nowadays, advanced techniques such as HPTLCand HPLCare widely used over TLC due to higher sensitivity, rapid separation and better resolution.

2. Melting point[27]:

The melting point (MP) is an important parameter that gives information regarding any structural changes in the organic compound. The prodrug formation is characterized by a change in melting point which is normally notably different from that of either pure drug or lipid. The MP of drug will be increased or decreased due to incorporation of lipid moiety. A technique like DSCis widely used to determine the MP of compounds.

3. Ultraviolet spectroscopy:

It is one of the preliminary spectroscopy techniques used to identify the changes in the absorption peaks that occur due to a change in molecular structure. The UV-visible spectrum for pure drug, phospholipid, physical mixture, and prodrug is recorded. The absorption peaks in physical mixtures usually appear at the same wavelength as observed in pure drug and phospholipid. The production of new bonds and recently launched neighbouring groups can be verified by changing peaks, which can be attributable to prodrug synthesis.

4. Fourier transform infrared spectroscopy (FTIR)[28]:

The prodrug formation is confirmed using FTIR by comparing theIRspectrum of prodrug with individual components and physical mixture. The spectrum of a prodrug is commonly different from particular components or physical mixture due to chemical interactions between drug and phospholipid which leads to the generation of new bonds.

5. X-ray diffraction (XRD)[29]:

X-ray diffraction analysis is also performed to confirm the formation of the drug-phospholipid conjugate. In X-ray diffraction pattern the crystalline drugs demonstrate characteristic intense peaks while phospholipid which is amorphous, shows wide peaks.Due to the prevalence of the both free drug as well as phospholipids, the physical mixtures get both sharp as well as wide peaks. The production of a drug-phospholipid conjugate is indicated by the absence or depletion in the intensity of sharp peaks.

6. Solubility studies:

The solubility is again one of the criteria used in the characterization of drug-phospholipid conjugate. The former drug-phospholipid conjugate will affect the solubility profile of the drug. The conjugation of drug with lipophilic moieties decreases solubility and increases the membrane permeability. The solubility studies are performed in water and buffer solutions of different pH values. An excessive amount of sample beyond saturation is added in vials containing different solvents and equilibrated in shaker bath at 37^0C for 24 hrs at controlled rpm. After completion of 24 hrs, a known volume of sample is withdrawn and the amt. of drug solubilized is determined by UV-visible spectroscopy.

Characterization of vesicles[30-34]:

1. Surface morphology:

The shape and size of pharmacosomes are altered by certain parameters such as purity of phospholipids, speed of rotation, method of preparation. Surface morphology can be studied by using SEM and TEM, etc technique.

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2. Drug content:
Fordrug content determination an equivalent amount of drug-lipid complex is measured and transferred to volumetric flask which containing solvent. Then flask is sonicated to achieve solubilization for 24 hrs. Finally, the solutions are diluted and drug content is determined using UV-visible spectroscopy or HPLC.

3. *In vitro* drug release study:
The equilibrium reverse dialysis bag technique is used to perform *in vitro* drug release study. Dialysis bag containing donor phase (an emulsion of drug, drug lipid complex) suspended in a vessel comprising of continuous phase outside and stirred. At definite time interval dialysis bag is removed and analysed for drug release. This method has certain advantages as the rise surface area available for donor and receiver phase and increased efficiency due to reduction in the number of steps.

4. *In vivo* characterization:
Specific study models were selected based on the expected pharmacological action of the drug in the pharmacosomes. For evaluating *in vivo* hepatoprotective activity, the effect of test pharmacosomes on animals against alcohol or paracetamol-induced hepatotoxicity can be observed.

5. Stability:
FTIR spectrum of drug lipid complex in solidified form is compared with FTIR spectrum of its micro-dispersion in water later lyophilization at different time intervals. This spectrum data tells about the stability of pharmacosomes.

Table no 2: Pharmacosomes prepared using different drugs, lipids and preparation method with comments[1]:

Drug	Lipid	Preparation method	Comments
Aceclofenac	Soya phosphatidylcholine	Solvent evaporation	Improved bioavailability of Aceclofenac
Diclofenac	Soya phosphatidylcholine	Solvent evaporation	Improved solubility of diclofenac
Aspirin	Soya phosphatidylcholine	Solvent evaporation	Controlled release of Aspirin
Rosuvastatin	Soya lecithin	Hand shaking	improved bioavailability of Rosuvastatin
Acyclovir	Phosphatidylcholine	Tetrahydrofuran injection	Improved solubility of Acyclovir
Ketoprofen	Soya phosphatidylcholine	Solvent evaporation	Increased solubility and dissolution of Ketoprofen
Ornidazole	Soya lecithin	Solvent evaporation	Showed sustained release of Ornidazole
Losartan	Losartan	Solvent evaporation	Improved bioavailability of
Furosemide	Soya phosphatidylcholine	Solvent evaporation	sustained release of
Geniposide	Phospholipid	Solvent evaporation	Improved lipophilicity, absorption and permeation of Geniposide

CONCLUSION:
Pharmacosomes is a stepping stone to improve delivery of drugs containing active hydrogen atom (-OH, $-NH_2$, -COOH,). In Pharmacosomes drug is bound to the lipid by covalent, van der Waal and hydrogen bonding. The drug-lipid conjugate (prodrug) is amphiphilic in nature and get self-assembled in vesicles in an aqueous medium. In contrast to conventional liposomes, Pharmacosomes are characterized by an unusually high drug loading, amenability to sterilization, higher *in-vitro* stability, and a low burst release. A large variety of drugs formulated as Pharmacosomes using different lipids and preparation techniques have shown improved physicochemical properties and pharmacokinetic performance of the drug.

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Research Article

pH Dependent Mucoadhesive *In-Situ* Gel Formulation Based on *Abelmoschus esculentus* as Sustained Release Carrier for Gastro-retentivity of Famotidine

Bhagyashri V. Aiwale*, Bharatee P. Chaudhari, Shivani H. Deshmukh, Vivekkumar K. Redasani
YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara, India.
***Corresponding author's E-mail:** bhagyashriaiwale1998@gmail.com

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ABSTRACT

The major goal of this work was to develop and assess a novel in-situ gel system for sustained drug administration using natural polymers. Okra gum was extracted from the fruits of Abelmoschus esculentus using acetone as a drying agent. The physical and chemical properties of dried okra gum, including pH, solubility, viscosity, moisture content, infrared study using FTIR, and crystallinity study using XRD, were assessed. The in-situ gel was created using the powdered dried okra gum. The pH dependent gelation approach was used to generate an in-situ famotidine gel using varying concentrations of okra gum and tamarind gum. The system makes use of polymers that go through a sol-to-gel phase transition when certain physico-chemical conditions change. Viscosity and in vitro drug release were all considerably affected by the concentration of gelling agents and release retardant polymers. The results showed that the pH ranged from 6.7 to 7.4 and that the drug content ranged from 83.74 to 94.82 %. The viscosity of sol and gel strength was increased with increase in the concentration of polymer, also drug release sustaining. At the end of 8 hours, the in vitro drug release from formulations comprising various amounts of okra gum and tamarind gum was sustained. In all formulations, the drug had a retardant release.

Keywords: Famotidine, Okra gum, Tamarind gum, Gastro retentive drug delivery, in situ gel.

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INTRODUCTION

The creation of regulated and sustainable drug delivery systems has received more attention over the past 30 years. The development of polymeric drug delivery systems has received a lot of research attention. In-situ gel creation has received a lot of interest nowadays. This is mostly due to the in-situ gelling system's significant advantages, which include convenience of administration and decreased frequency of administration and assist to promote patient compliance [1]. The ability to give regulated drug delivery with improved gastro retention within the stomach is provided by gastro retentive in situ gelling systems, also known as stomach-specific systems. In situ gelling systems are liquid at room temperature but undergo gelation when in contact with body fluids or change in pH [2]. Since the gel produced by the in-situ gelling technique is lighter than gastric fluids, it floats above stomach contents or adheres to the gastric mucosa because of the bio adhesive nature of the polymer. This prolongs the time the dosage form spends in the stomach and causes gastric retention, which in turn extends the duration of time the drug is delivered to the gastrointestinal tract. The system makes use of polymers that undergo sol–gel phase transition owing to changes in specific physicochemical parameters. Several polymers are used to form in situ gel, including tamarind gum, xyloglucan, pectin, gellan gum, and sodium alginate [3,4].

Famotidine, histamine is a H2-receptor antagonist that prohibit gastric secretion both locally and systemically, is used to treat gastric ulcers. The dose frequency is twice or three times day and may vary from person to person. Famotidine is rapidly and incompletely absorbed from gastrointestinal tract with the bioavailability of about 45% having an elimination half-life (t1/2) of 3 hours [5]. The use of natural bio-degradable polymer okra gum and tamarind gum was used for this purpose at various combinations in present work. Trisodium citrate, which is a component of the formulation, aids in keeping it liquid until it reaches the stomach. When the formulation enters the stomach, the presence of an acidic environment causes Ca++ to be released, which causes the formulation to gel. The buoyancy of the in-situ gel is maintained to extend period of time due to the release of carbon dioxide in the stomach pH.

Therefore, the goal of the work was to create an in-situ gelling system containing famotidine utilising okra gum and tamarind gum by a pH dependent gelation method, and to assess its physicochemical properties including measurement of pH, viscosity, gelation time, in vitro release characteristics and drug content.

MATERIALS AND METHODS

Famotidine was gift sample from yarrow chem. Pvt. Ltd, Mumbai. Okra gum is extracted in laboratory and

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Tamarind gum was obtained from Chhaya industries, Barshi and all other chemicals used were of analytical grade.

Extraction of Okra gum

The fresh *Abelmoschus esculentus* fruits were collected and washed with water. The fruits were crushed and soaked in water for 5–6 hrs, boiled for 30 min and left to stand for 1 hr to allow complete release of the mucilage. The mucilage was precipitated by adding acetone after being separated using a multi-layer muslin cloth (three times the volume of filtrate). The precipitate obtained was collected, dried in an oven at 40°C, and passed through a sieve #80 to obtain discrete powder [6].

Characterization of Extracted Okra Gum [7]

Experiments were carried out in accordance with British Pharmacopeia 2007 and altered based on prior studies.

Solubility Test

Stirring 10 mg of okra powder in 10 mL of water, acetone, chloroform, and ethanol to qualitatively assess the extracted gum's solubility (1 % dispersion). Visual examination of the solute was used to determine solubility.

pH Determination

pH metre was used to determine the pH of the sample's 1 % W/V dispersion in water after it was stirred continuously for 5 minutes.

Viscosity

Viscosity of Okra gum at 1% and 0.5% concentrations was performed using the Brook-field digital viscometer.

Moisture content

Moisture content of okra gum powder was conducted by measuring 100mg of powder using hot air oven with loss on drying at 105˚C.

Fourier Transform Infrared (FTIR)

The Fourier transform-infrared (FTIR) spectrum of the sample was recorded in FTIR Thermo Scientific range between 400–4000 cm^{-1}, in attenuated reflection mode (ATR).

X-Ray Diffraction Analysis

X-Ray diffraction was carried out on Bruker D8 Advance instrument at 250 exposures.

Pre formulation Studies

FTIR Spectroscopy of Famotidine

FTIR spectroscopy was carried out to check the compatibility between drug and polymer. The usual FTIR spectrum of the pure drug was compared to the FTIR spectra of the drug with polymers [8].

UV Spectroscopy

10 mg of famotidine transferred into 100 ml volumetric flask. 0.1 N HCL was used to get the volume assigned to 100 ml (stock-1) by using UV visible spectrophotometer in the scale of 200-400 nm UV spectrum was recorded[9].

Calibration curve of famotidine in 0.1 N HCL

50 mg of famotidine was dissolved in 50 ml of 0.1 N HCL. The solution was then diluted with 0.1 N HCL to obtain 2, 4, 6, 8, 10 and 12 µg/ml solution. It was then measured by UV visible spectrophotometer at 265 nm [10].

Melting Point

Melting point equipment was used to find out the melting point of Famotidine. Drug was placed in a glass capillary with flame-sealed end to determine melting point. Inside the melting point apparatus, which had a magnetic stirring facility, the capillary containing the drug was submerged in liquid paraffin.

Preparation of in situ gelling Sols

The in-situ gel formulations of F1 to F3 was prepared by using okra gum and tamarind gum was 69eionize in F4 to F6 formulation. The polymer solutions (sodium alginate, tamarind gum and okra gum) of various concentrations were prepared by adding to 69eionized water containing 0.17% w/v trisodium citrate and heated to 90°C with continuous stirring. After cooling to below 40°C appropriate amounts of calcium carbonate (0.05% w/v), drug solution of famotidine and preservative (methyl paraben) was added to the polymer solution and volume was adjusted to 20 ml with distilled water. The mixture was stirred by using a magnetic stirrer to ensure thorough mixing (Table 1) [11].

Table 1: Composition of the in-situ gelling formulation

Sr.no	Ingredient	F1	F2	F3	F4	F5	F6
1	Sodium alginate (%W/V)	1	1.5	2	1	1.5	2
2	Okra gum (%W/V)	0.2	0.4	0.6	-	-	-
3	Tamarind gum (%W/V)	-	-	-	0.2	0.4	0.6
4	Trisodium citrate (%W/V)	0.17	0.17	0.17	0.17	0.17	0.17
5	Calcium carbonate (%W/V)	0.05	0.05	0.05	0.05	0.05	0.05
6	Famotidine (mg)	40	40	40	40	40	40
7	Preservative (%W/V)	0.2	0.2	0.2	0.2	0.2	0.2

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Characterization of the in-situ gel formulations

Determination of the visual appearance

All the preparations were visually inspected for their appearance, clarity, and consistency.

Measurement of the pH

A calibrated digital pH metre was used to measure the pH of each formulation. For each formulation, the readings were held three times, and the averages of the readings were held into consideration[12].

In vitro gelation study

The gelling capacity was determined by placing 10 ml of solution in 100 ml of stimulated gastric fluid (pH 1.2) freshly prepared and equilibrated at 37 ± 0.5°C. When the formulation came in contact with the gelation medium, it was quickly converted into a gel-like structure. The in vitro gelling capability was analyzed based on the gel's stiffness and how long it maintains its stiffness.

According to the period of time the created gel required and how long it lasts, the in vitro gelling capability was primarily categorised into three groups [10].

• (+): Gels in few second and disperse immediately.

• (++): Immediate gelation does not disperse rapidly.

• (+++): Gelation after few minutes remains for extended periods.

Determination of viscosity

The viscosities of the formulations were measured using fresh samples three times using a Brookfield digital viscometer with an S21 spindle at 50 rpm. The average reading was taken after each measurement.

In vitro buoyancy study

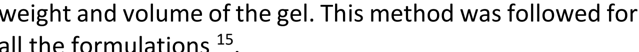
The in-vitro buoyancy study was carried out using stimulated gastric fluid (0.1N HCl, pH 1.2). 37 ±5°C was maintained as the medium temperature. In the dissolution media, 10 ml of the in-situ gel formulation were added. The time taken by the in-situ gel formulation to reach the medium surface (floating lag time) and how long it remained buoyant (the floating duration) was noted [1,13].

Determination of the drug content

80 ml of 0.1N HCl, pH 1.2, was combined with 5 ml of the formulation corresponding to 10 mg of the drug, and the solution was stirred for one hour in a magnetic stirrer. The solution was filtered and diluted with 0.1N HCl, pH 1.2, after 1 hour. The drug concentration was then determined by ultraviolet (UV) visible spectrophotometer at 265 nm against a suitable blank solution [14].

Measurement of density of gel

30 ml of the in-situ formulation was poured into a beaker containing 50 ml of 0.1N HCl. 10 ml of the gel formed was elevated in measuring cylinder and weight of the gel was measured. The density was determined using both the



weight and volume of the gel. This method was followed for all the formulations [15].

Measurement of gel strength

30 g of the gel was elevated in a 50 ml beaker and a 50 g weight was placed on the centre of the gel surface and allowed to penetrate through the gel. The time taken by the 50 g weight to penetrate 5 cm down through the gel was noted for all the formulations. The same method was followed for 3 times for each fresh formulation and average time was noted [16].

In vitro drug release studies

A USP dissolution equipment (Type II) with a paddle stirrer operating at 50 rpm was used to assess the drug release from the formulations. This slow speed is necessary to avoid breaking of the gelled formulation. The dissolution medium was 900 ml of the simulated gastric fluid (0.1N HCl, pH 1.2), and the temperature was kept at 37±5°C. In situ gel was formed when 10 ml of the formulation were added to the dissolution vessel without disturbing the dissolving medium. At each time interval, 3 ml of the sample was withdrawn and replenished with fresh medium. The samples were collected, filtered, and suitably diluted before being analysed at 265 nm with a UV spectrophotometer [17].

***In vitro mucoadhesive study* [18,19]**

Using a modified bioadhesion test equipment, the force necessary to separate each formulation from goat tissue was measured in order to estimate the mucoadhesive strength of each formulation.

Modified bioadhesion test apparatus

Modifying the double beam physical balance as shown in figure 1 can create the bio adhesion test apparatus. the two pans of the physical balance were removed. A light-weight plastic glass was used to replace the right-hand pan, and a glass vial was suspended from a strong thread on the left-hand side of the balance, with the height of the vial and it adjusted to allow for a lower vial to be placed beneath it. The two sides of the balance were adjusted so that the right side weighed 5 g more than the left. To determine the bioadhesive strength, a piece of goat mucosa was cut and utilised as a membrane. It was attached to both glass vials using a thread after being properly washed with physiological saline solution so that both mucosal surfaces were exposed on the outsides of the vial surfaces (figure no.1). The buffer pH 5.5 was added to the jacketed glass container, which was kept at a constant 37˚C±1˚C. The vial was then placed inside of it. The membrane was kept at this temperature for 30 minutes to allow for equilibration. the jacketed glass container containing beaker was kept below the right-hand setup of the assembly. The gel was stuck to the lower side of the beaker as a thin layer. The assembly was kept undisturbed for 1 min and the weights were slowly added to the left-hand side till the membrane surface just detached from the gel surface just detached from the gel surface. The excess weight on the left-hand side, i.e., a measurement stress in dyne/cm2 was determined from the

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minimal weights that detached the tissues from the surface of each formulation using the following equations: total weight (g) minus blank (weight in gm required to detach mucosal surface without gel layer).

Detachment stress (dyne/cm^2) = m x g/A

Where, m = weight required for detachment for two vials in grams

g = acceleration due to gravity [980cm/s^2]

A= Area of tissue exposed

The goat mucosa was changed for each measurement. For each of the gel formulations, measurements were carried out times.

Stability study

The optimized formulation of in situ gel were placed in an amber colour bottle with aluminium cap as a closure. It was tightly sealed. The stability study was carried out for 1 month. Stability of the in-situ gel formulation was monitored at room temperature (25˚C+2°C). Samples were periodically removed and evaluated for viscosity, drug content, pH and in vitro release [20].

RESULTS AND DISCUSSION

FTIR and compatibility studies

All the characteristic peaks of Famotidine were present in the spectrum of drug and polymer mixture, indicating compatibility between drug and polymers. The spectrum confirmed that there is no significant change in chemical integrity of the drug.

Determination of λ $_{max}$ of Famotidine

λ $_{max}$ of Famotidine was determined by utilised stock solution and analysed spectroscopically at 265nm wavelength.

Calibration curve of Famotidine

The absorbance of the solution was recorded at 265 nm by using UV visible spectrophotometer. 0.1 N HCL was taken as blank. The graph of absorbance vs. concentration was shown to be linear in the famotidine concentration range.

Melting point determination

By using a melting point equipment, the melting point of famotidine was detected to be between 160-162˚C. The reported melting point range for famotidine 163-164˚C.

Characterization of Okra Gum

Solubility test

Okra powder was shown to be sparingly soluble in water and insoluble in acetone, ethanol and chloroform. An increase in solubility was observed when temperature was applied.

pH Determination

The pH of Okra gum is 6.59.

Viscosity

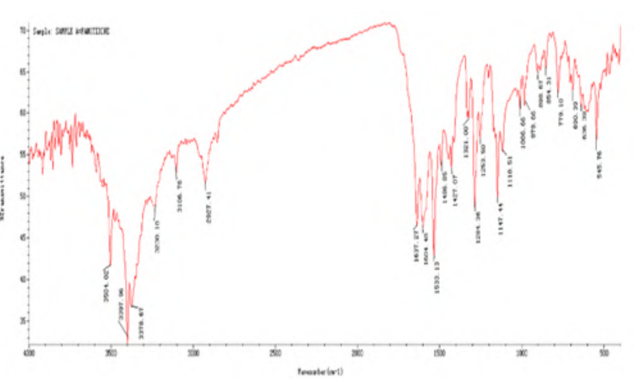
Viscosity of Okra gum 1% solution is higher (228.78cps) compared to the viscosity of Okra gum at a lower concentration (0.5% solution) which is 62.32 cps. This indicates that Okra gum has higher viscosity at a higher concentration.

Moisture content

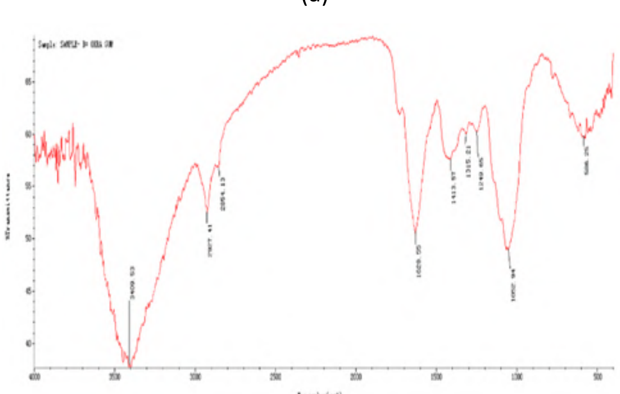
Moisture content of Okra gum is 14.83%, indicating that Okra gum contains bound moisture to the polymer. This is due to the polymer adsorption sites that is able to bind water molecules to the polysaccharide structure via hydrogen bond [9], which leads to a larger permeability of hydrophilic materials.

FTIR and compatibility studies of Okra gum

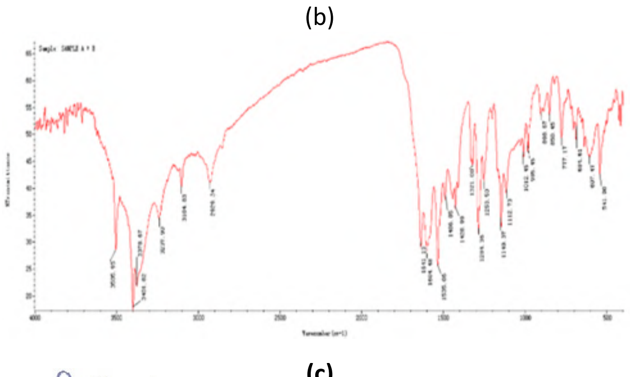
No considerable changes in the IR peaks of the extracted Okra gum as shown in figure 1.



(a)



(b)



(c)

Figure 1: FTIR peak of Famotidine (a), Okra gum (b) and famotidine with okra gum (c)

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X-Ray Diffraction Analysis

XRD analysis of Okra as can be seen in Figure 2 showed that it consists of crystalline structure. The sharp peak that could be seen from the X-ray diffraction spectrum indicates the crystalline nature of the polymer.

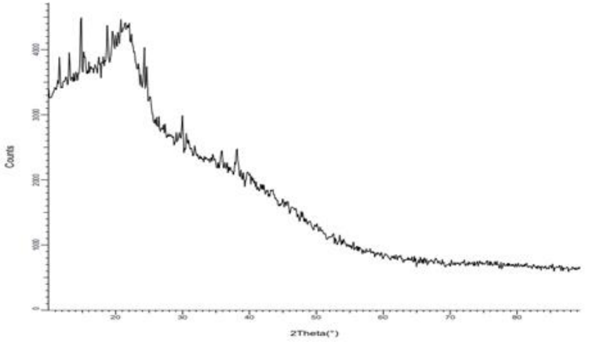

Figure 2: X-ray diffraction analysis of Okra gum

Characterization of the *in-situ* gel formulations

Visual appearance

Visual appearance was evaluated on all of the formulations. The results are shown in Table 2 for the developed formulations of in situ gel containing okra gum, which had a light brownish appearance, and tamarind gum, which had an off-white appearance. The formulations were free running and did not produce any gelation at room temperature.

pH measurements

According to Table 2, the pH of each formulation was determined to be adequate and ranged from 6.7 to 7.4. The pH of each formulation was within the orally acceptable range.

In vitro gelation study

The gelation study was conducted in 0.1N HCL, pH 1.2. On an ordinal scale between + and +++, the gelation properties of the formulations were evaluated as given in table no.2. All the formulation on contact with the gelation medium had undergone sol to gel transition. It was detected that the gel intensity was increased when the concentration of polymers was increased. Table 2 has shown that the formulation F1, F3, F5, F6 were satisfactory to cause gelation.

Table 2: Appearance, pH, Gelling capacity, Pourability

Formulation code	Appearance	pH	Gelling capacity	Pourability
F1	Light brownish	6.7±0.02	+	Easily pourable
F2	Light brownish	7.1±0.06	+++	Easily pourable
F3	Light brownish	7.3±0.03	+++	Easily pourable
F4	Off -white	7.2±0.07	++	Easily pourable
F5	Off -white	6.9±0.01	+++	Easily pourable
F6	Off -white	7.4±0.04	+++	Easily pourable

Table 3: Viscosity, Floating lag time, Floating duration, Percentage drug content

Formulation code	Viscosity (cps)	Floating lag time (s)	Floating duration (hr)	Percentage drug content (%)
F1	69.20±0.02	22	5	88.16 ± 0.34
F2	85.52±0.16	19	7	83.74 ± 0.45
F3	90.60±0.45	16	8	91.46 ± 0.53
F4	82.34±0.49	26	11	84.87 ± 0.23
F5	88.68±0.25	31	12	89.72 ± 0.41
F6	108.52±0.65	33	12	94.82 ± 0.59

In vitro buoyancy study

The floating lag time is the duration of time that the formulation required to appear on the medium's surface and the floating duration is the period of time that the formulation floated constantly. Buoyancy studies results are given in Table 3. A gel barrier forms on the plane of the formulation when it comes into contact with an acidic environment due to the calcium ions' cross-linking and gelation processes. The formulation floats because the carbon dioxide emitted is trapped in the gel matrix. The dispersing of carbon dioxide and drug release are then further constrained by the polymeric network. The floating capability of the formulations mainly depends on concentration of the gelling polymer, carbon dioxide and cation source. The formulation containing okra gum (F1-F3) is less floating lag time but more floating lag time containing tamarind gum (F4-F5).

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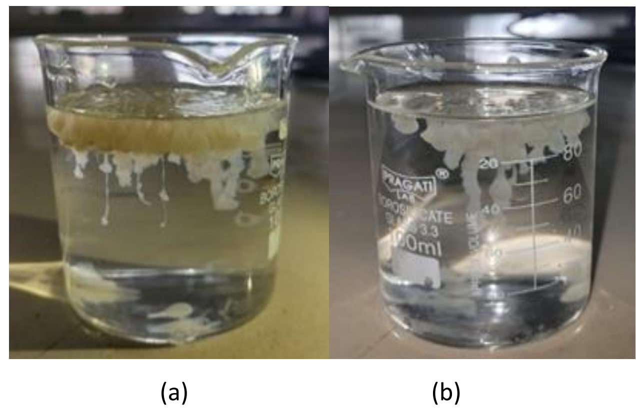

(a) (b)

Figure 3: *In vitro* buoyancy study of (a) Okra gum and (b) tamarind gum

Viscosity

The viscosity of all the in-situ gelling formulations determined at 50 rpm using Brookfield digital viscometer. The results of viscosity measurement of each formulation are shown in Table 3. The increase in viscosity of the formulations that were observed with the increase in the concentration of polymer can be related to the increasing crosslinking of the polymer. Okra gum-based formulations have greater viscosities, which results in in situ gel and slower drug release. The all formulations of viscosity range between (69.20±0.02 and 108.52±0.65).

Drug content

Drug content is one of the important evaluation parameters for any type of dosage form. The percentage drug content of each formulation was between the range of 83.74-94.82 indicating uniform distribution of drugs in all formulations as per monograph Table 3.

Measurement of density of the gel

Regarding the gastro retentive dosage form's ability to float, density is an important evaluating parameter. The formulation must have a density that is less than or equal to the gastric content's density (1.004 gcm^3) in order to float on them. The density of each formulation given in (Table 4) has density less than the above-specified value. As a result, the floating of the gastro retentive in situ gel is promoted in the stomach.

Measurement of gel strength

All the formulations showed good gel strength in which okra gum as compare to tamarind gum ranges are same from 18.7s to 30.3s. This says the increase in polymer concentration causes an increase in gel strength (Table 4) gives the gel strength of all the formulations.

Table 4: Density and gel strength of the in-situ gel formulation

Formulation code	Density (g/cm^3)	Gel strength (s)	Mucoadhesive strength ($dyne/cm^2$)
F1	0.422±0.36	18.7 ±0.06	1191±33.41
F2	0.501±0.45	24.2±0.12	1275±33.13
F3	0.554±0.42	29.8±0.23	1511±33.41
F4	0.482±0.54	22.5±0.33	785.6±18.02
F5	0.526±0.56	26.9±0.45	915.1±18.02
F6	0.579±0.62	30.3±0.56	1011±21.31

Table 5: Stability studies of in situ gel formulation

Days	pH	Viscosity	Drug content (%)	Drug release (%)
Initial	6.7±0.02	69.20±0.26	88.16 ± 0.34	90.74
After 1 month	6.7±0.06	69.20±0.26	88.16 ± 0.34	90.74
After 2 months	6.6±0.14	67.28±0.35	87.22 ± 0.54	89.35
After 3 months	6.5±0.11	69.28±0.12	88.11 ± 0.13	88.22

***In vitro* drug release study**

The in vitro drug release studies, it was mentioned that the release of the drug from the prepared gastro retentive in situ gel reduces as the concentration of the gelling agent increases. The effect of polymer concentration on in-vitro drug release from in situ gels. The plot of % cumulative drug release v/s time (in hours) was plotted and depicted as shown in Figure no.10. Drug releasing pattern of various formulation contains a different concentration of gelling agent and drug release retardant polymers are given as follows: Okra gum: F1 > F2 > F3 and Tamarind gum: F4 > F5 > F6 as shown in Figure 4. The percentage drug release from formulations containing various concentrations of Okra gum at the end of 8 hrs was observed to be 90.74%, 86.24%, and 82.31%, respectively, for F1, F2, and F3. Similarly, percentage drug release from formulations containing various concentrations of Tamarind gum at the end of 8 hrs was observed to be 91.82%, 88.16%, and 83.74%, respectively, for F4, F5, and F6. The retarded release observes in above formulations.

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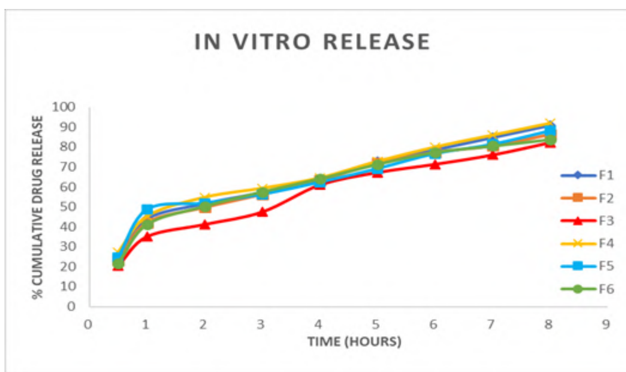

Figure 4: *In vitro* drug release

Mucoadhesive strength

Mucoadhesive strength of the in-situ gel formulation plays major role in giving the idea about gastric residence time of the formulation. Formulation must have enough mucoadhesive property so that it will remain in GIT for longer the gastric absorption of drug. The formulation containing okra gum (F1-F3) is more mucoadhesive strength but tamarind gum containing (F4-F6) less mucoadhesive strength. Result of mucoadhesive strength study is given in Table 4.

Stability studies

The stability study of optimized formulation F3 was carried out for 3 months at room temperature and humidity condition. Stability study's results designated that the there was no significant change in the pH, viscosity, drug content (%) and drug release (%) as shown in Table 5.

CONCLUSION

In the present study in situ gel of famotidine were produced by using different concentration of okra gum and tamarind gum to improve its oral bioavailability and sustained release activity. Okra gum shows less floating lag time and more mucoadhesive strength but tamarind gum shows more floating lag time and less mucoadhesive strength. Both okra gum and tamarind gum show result but okra gum shows significant result as that of tamarind gum. Based upon obtained results it concluded that prepared formulation is satisfactory for clinically use of famotidine and formulation of in situ gel using okra gum was successfully prepared.

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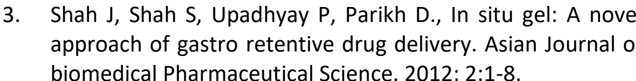

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Regulatory Intelligence

Neha Nangare[1], A B. Velhal[2]

Abstract: ***Regulations are a common way for governments to exert control over the activities of individuals, businesses, and communities in order to promote the common good. Regulations can be for any area of society, such as environmental wellness, such as water or air quality, public health, or data privacy for internet safety. They can, however, be produced by various entities with overlapping jurisdictions, resulting in widespread confusion, misunderstanding, and inaction. AI has the ability to play a significant role in assisting various stakeholders in better understanding existing regulations, their gaps and duplications, and recommending methods to strengthen them in order to streamline decision-making. Regulation Intelligence is the term we use to describe the difficulty of enabling improved comprehension of regulations. Pharmaceutical companies all across the world have long struggled with the massive amount of data they must manage. These can be described as the twin issues of manually researching changing regulatory requirements to ensure better compliance and decreasing rework as a result of departmental silos within the business and the lack of readily available historical information. These two difficulties result in lower operational efficiency, as well as more time, effort, and expense. This paper examines how these issues might be addressed comprehensively by adopting a technology-centric approach to developing a smart regulatory compliance solution. This solution will be able to deliver actionable insights and support precise, easily accessible, and contextual information, allowing for on-demand access to literature.***

Keywords: Regulatory intelligence

1. Intelligence on regulatory issues

Regulatory intelligence, in general, refers to the monitoring, collection, and analysis of publicly available and experience-based regulatory information in order to develop strategies for more time and cost-effective drug development.

Regulatory intelligence professionals provide strategic information to the drug development process, act as liaisons with regulatory bodies, and distribute information to the right stakeholders. Kirsten Mesmer and Charity – Anne Schuller, regulator experts, present an overview of applicable delivery methods and general considerations for communicating information via spreadsheets, text documents, slide presentations, strategy reports, and competitive intelligence reports in " Regulatory Intelligence Communication for Business Impact. "

The authors discuss how to get the most out of regulatory information when responding to specific stakeholder requests, as well as communication tips.

Regulatory Intelligence enables regulatory professionals to determine the requirements for global clinical trials, compliance procedures, manufacturing requirements, advise personnel, answer strategic regulatory questions, and develop a global marketing application using data from regulatory intelligence. Going deeper into this blog will give you a better understanding of what regulatory intelligence is and how it operates.

However, three points should be remembered if you want to grasp the essence of RI:

1) Collect information
2) Regulatory strategy
3) Information

1) Collecting information

Regulatory specialists used to limit RI activity to this issue solely at one point. When gaps in the input and output were discovered, it was clear that several critical facets were missing from the shelf. That's when the rest of the puzzle fell into place.

To begin, RI experts conduct extensive study into regulatory requirements for a certain product in a specific geography. There are many sites that RI experts use to consolidate their research material when it comes to obtaining appropriate regulatory information. These are some of the resources available:

a) Regulations on Websites, Blogs, and Social Media Groups
b) Seminars and Training Sessions-Professional Newsletters-Competitor Product Analysis
c) literature
d) Requests for Information (FOIA)
e) E-mails pertaining to regulatory issues – Networking
f) Paperwork
g) Messages

2) Knowledge

Because the initial phase contains a large amount of research material, it is clear that this data must be filtered in order to acquire useful information for the objective. You can think of it as jigsaw pieces, and now we need to make sure that all of them fit together to acquire what we need. An effective regulatory strategy conveys the best answer and fosters adequate planning throughout an organization's numerous disciplines, from manufacturing to marketing.

This task entails keeping track of things like the regulatory industry's current trends and patterns. We've been emphasizing that in order for RI to be effective, it must stay up with the most recent changes in regulations and guidelines. As a result, it becomes clear that this method may undergo several alterations in order to eliminate the required conclusion.

Knowledge of the sector and its history, as well as soft skills, are required of regulatory intelligence specialists. In regulatory affairs and the pharmaceutical and/or medical

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device industries, there are no hard-and-fast regulations concerning how many years of experience a new worker should have. However, a reasonable rule of thumb for entering the regulatory intelligence field is that entry-level positions should demand a minimum of 5 years in industry and 3 years in regulatory affairs. The number of years of experience necessary rises in proportion to the position's seniority.

3) Action Plan for Regulation

The main goal of the aforementioned tasks is to develop the most appropriate and realistic regulatory strategy for a company. In different countries, different products have distinct regulatory rules. This is why experts recommend a plan of action that lays out a strategy for implementing regulatory actions in the target distribution markets. However, this strategy never results in a completed work. It keeps moving forward as the regulatory space's mandates change.

Importance of Regulatory Intelligence:
- Provides regulatory professionals with information to:- identify opportunities
- More indications and more precise pre-clinical and clinical development programmes
- Quicken development/improve efficiency

a) Recognize potential pitfalls
- Issues with compliance, as well as changes in the requirements for certain indications

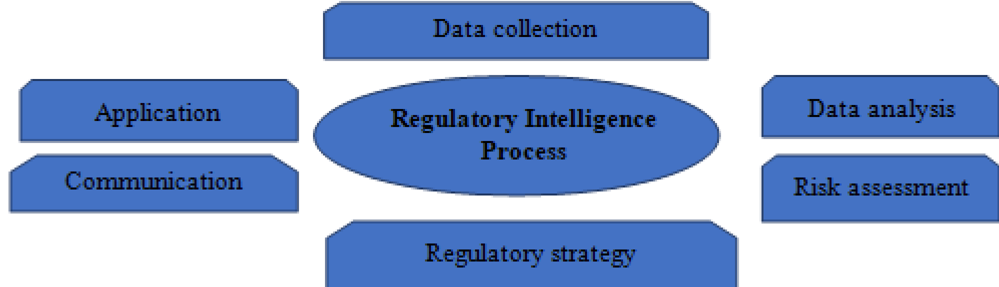
b) Answer specific development questions posed by team RI-Predict review times for product and/or update to product Provide?
- Research for product teams
- Supports execution plan
- Policy comments to shape legislation
- Track legislation
- Track approvals, non-approvals, and withdrawals
- Knowledge management
- Training
- Corporate policy creation

The RI procedure

The practice of delivering strategic information that underpins the making of effective and efficient decisions in relation to the regulatory aspects of the business is known as regulatory intelligence.

The following activities are included in the procedure:

Selection of relevant publicly available data sources;
- Data collection;
- Data analysis;
- Generation of significant information for the definition of the regulatory strategy based on the analysis;
- Communication of the implications of this information for the business;
- Continuous monitoring of the regulatory environment, looking for opportunities to model future regulations, policies, and legislation.



In medication development, what role does regulatory intelligence play?
- Regulatory intelligence professionals provide strategic information to the drug development process; act as liaisons with regulatory bodies, and channel information to the right Ate stakeholders.
- Kirsten Messmer and Charity-Anne Schuller, regulato-Ry experts, present an overview of applicable Delivery methods and general considerations for communicating information via spread-Sheets, text documents, slide presentations, strategy reports, and competitive intelligence Reports in "Regulatory Intelligence Communication for Business Impact. "
- Authors discuss how to get the most out of regulatory information when responding to specific stakeholder requests, as well as communication tips.

Regulations are evolving at a faster rate than ever before –

It's necessary to be on the ball all of the time.
- New technology and goods, such as the world's first 3D-printed medication Approved recently it's possible that it won't fit well in the current regulatory context, necessitating careful adaption.

Harmonization and Expansion
- Australia is constantly implementing new EU legislation – nations may join the EU – Increased transparency equals increased accountability.
- Recent drive for transparency in the EU and the US – for example, trial registrations – More information becomes publicly available – Information overload

In pharmacovigilance, what role does regulatory intelligence play?

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QVigilance continuously monitors regulatory information from local, regional and global authorities and organisations for pharmacovigilance related regulatory intelligence to ensure that we and our customers are always up to speed and thereby maintain compliance with the latest regulatory requirements and guidelines;

1) Drug safety
2) That is dependable
3) Scalability should be improved.

The act of acquiring and evaluating publically available regulatory information, communicating the consequences of that information, and monitoring the present regulatory environment is known as Regulatory Intelligence in Pharmacovigilance (PV).

Regulatory intelligence is the process of staying current with new regulatory standards as they are enacted by governments and regulatory agencies. These regulations apply to both pharmaceutical drugs and medical equipment that are in development and have been approved for sale. This means that new or altered PV-relevant regulatory material must be examined and assessed on a regular basis for potential influence on corporate operations and pharmacovigilance strategy. Regulatory Intelligence efforts must be reported to stakeholders, and an effect assessment must be done and documented.

PV Regulatory Intelligence is managed by ProPharma Group for a number of clients. Regulatory Intelligence is also used by our team to keep our own internal knowledge current, such as that of QPPVs (Qualified Persons for Pharmacovigilance), LPPVs (Local Persons for Pharmacovigilance), and others.

Regulatory intelligence sources and communication.

- Regulatory intelligence sources vary by company. Smaller enterprises must rely on public regulatory intelligence sources, but larger, better-resourced companies can obtain rights to paid subscription services like Cortellis or Tarius.
- Regulatory authority websites were cited as the most popular source of regulatory intelligence by survey respondents, which is understandable given that they are the best source of regulatory information.
- It's worth noting that the 2019 poll results showed less use of subscription services than earlier versions of the survey.
- This could indicate that there is more free information on the internet, reducing the need to pay for high-quality regulatory intelligence.

RI in action:

1) Programmed optimization
2) Clinoptimization's Possibility
3) Adjustment of the development plan
4) Questions and answers, as well as a review of regulatory requirements
5) Regulatory overview preparation
6) Contracts for research bidding
7) Internal and external education and training companies Alerts that are specific to your needs, as well as a newsletter
8) Sometimes it's as simple as seeing if a particular medicine is available in other nations.

2. Conclusion

Pharmaceutical companies may function more efficiently and respond quickly to any developing urgent scenario by reorganising outdated procedures and reinventing regulatory information with the help of digital technologies such as artificial intelligence. The integrated Regulatory Intelligence solution offers a more simplified ow of global regulatory requirements by facilitating the reuse of internal data. As a result, the necessity of the hour is to imagine a connected future using digital technologies and RI. Its diverse capabilities and potential can assist pharma companies in overcoming important issues and achieving their goal of becoming a smart firm.

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DRUG DEVELOPMENT PROCESS

MISS.RITU BHOSALE ,MR.A.B.VELHAL ,DR.V.K.REDASANI
STUDENT , PROFESSOR , PRINCIPAL
DEPARTMENT OF B PHARMACY
YSPM'S YASHODHA TECHNICAL CAMPUS , SATARA , INDIA

Abstract: Drug discovery is a procedure that tries to find a therapeutically beneficial chemical for curing and treating disease. Identification of candidates, synthesis, characterization, validation, optimization, screening, and tests for therapeutic efficacy are all part of this process. Once a molecule has been proven to be significant in these studies, it will begin the medication development process prior to clinical trials. To create a medicine that is safe, effective, and meets all regulatory standards, the new drug development process must go through numerous stages. One of the main points of our article is that the process is long, complicated, and expensive enough that multiple biological targets must be considered for any new treatment that is eventually approved for clinical use, and new research methods may be required to investigate each new target. It takes a long time and a lot of effort to turn a discovery into a commercial medicine. It takes roughly 12 to 15 years from discovery to licensed drug, and an expenditure of about $1 billion is required. A million molecules are screened on average, but only one is investigated in late-stage clinical trials and eventually made available to patients. This article gives a quick overview of how new drugs are discovered and developed.

***Index Terms* - Drug discovery , Development , Validation, Optimization , Screening.**

I. INTRODUCTION

Drug discovery is a multidimensional process that include identifying a drug molecule that is therapeutically useful in the treatment and management of a disease. Typically, researchers discover novel medications by developing new perspectives on a disease process that allow researchers to construct a medicine to counteract or stop the disease's symptoms. The identification of drug candidates, synthesis, characterization, screening, and assays for therapeutic efficacy are all part of the drug development process. Following clinical trials, if a molecule achieves favorable findings in these investigations, it will begin the process of medication development. Due to the high costs of R&D and clinical trials, drug discovery and development is a costly process.(1)

A single new medicine molecule takes almost 12-15 years to develop from the moment it is discovered to the time it is accessible on the market for treating patients. For every 5,000-10,000 compounds that join the research, Success necessitates vast resources, including the best scientific and logical brains, cutting-edge labs and equipment, and multidimensional project management. Persistence and good fortune are also required. Drug discovery eventually offers hope, faith, and relief to billions of sufferers(2)

II. STAGES OF DRUG DISCOVERY

stage 1: Target identification:

Target identification is the first and key step in the drug discovery channel. A drug target is the specific binding site for drug in vivo through which it exerts action. Usually, drug target refers to a single biomolecule.(4)

A drug target can be an established drug for which there is good scientific Know-how which is supported by publications that describe both how the target behaves in normal physiology and how it is involved in human pathology There are many drugs targeting established drug targets. A drug target can also be potential drug targets which are biomolecules whose functions are not fully understood and which lack drugs targeting them.(5)

A drug target has any of the following characteristics

1. the drug targets can be a biomolecule(s) such as a protein that could exist in solitary or complex forms.
2. The biomolecules have specific locations or sites that match with the other, the drug
3. The structure of biomolecules may change when it binds to drugs. The changes in structure are usually reversible.
4. Various physiological responses occur when the structure of a biomolecule changes, causing the cell, organ, tissue, or body condition to be regulated.(5)

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Stage 2: Target Validation:

Target validation is the basis for new drug exploration in the process of drug discovery. Target validation helps to new drug research and development and to provide more insight into the pathogenesis of target-related diseases. (6)

The target validation process includes

1 Discovery of the biomolecule of interest

2. Evaluation of its potential as a target.

3. Designing a bioassay to measure its biological activity.

4. Constructing a high-throughput screening method. [6]

Stage 3: Lead Discovery

Leads can also be obtained by molecular modelling assisted by 3D computer graphics, which allows the development of structures based on new and pre-existing molecules to increase desired features while eliminating undesirable properties to develop highly selective targeted compounds. A combinatorial chemistry wherein unplanned mixing and matching of large numbers of chemical building blocks to produce libraries of all possible combinations can also be attempted to get leads. This technique generates billions of compounds, screened by high-throughput screening (HTS), meaning thousands a day. [3]

Stage 4: Lead optimization:

Lead optimization is a procedure that begins with the identification of a compound that has the potential to have a biological effect and ends with the selection of the best compound. Molecules are chemically modified and described to produce molecules with desirable qualities, which are then converted into drugs. Physiochemical qualities, pharmacokinetic properties, and toxicological elements of leads are optimized in vitro and in vivo for efficacy and potency[7]

Stage 5: Pre-clinical and Clinical Development:

Companies use stylized statistics to illustrate the risks in preclinical research, such as that on average, only one in every five thousand compounds that enters drug discovery to the stage of preclinical development becomes an approved drug[8]

Clinical trials are organized by the National Institutes of Health (NIH) into 5 different types:

1. Treatment trials: This trial tests the experimental treatments or a new combination of drugs.

2. Prevention trials: This trial looks for ways to prevent a disease or prevent it from recuring.

3. Diagnostic trials: This trial finds better tests or procedures for diagnosing a disease.

4. Screening trials: This trial tests methods of detecting disease.

5. Quality of life trial: This study looks into ways to improve the comfort and quality of life for those who have a chronic illness[12]

III. PRE-CLINICAL STUDIES

Preclinical development, also known as nonclinical studies, is a stage of drug development that occurs before clinical trials (human testing) and collects essential feasibility, iterative testing, and drug safety data, generally in laboratory animals.[9]Preclinical studies are used to determine a starting, safe dose for first-in-human studies and to assess the product's potential toxicity, which often includes new medical equipment, prescription medications, and diagnostics.[8]

The pre-clinical development includes developing a method of large-scale synthesis, animal safety studies, carcinogenicity tests, drug delivery, elimination and metabolism studies , drug formulation experiments, a dose-ranging studies in animals. At this stage, wide range dosages of the potential drugs are introduced to the cell line or animal to get preliminary effectiveness and pharmacokinetic information[8]

IV. CLINICAL STUDY

• A clinical study is a research project that uses human volunteers (also known as participants) to further medical knowledge. Clinical trials (also known as interventional studies) and observational studies are the two basic forms of clinical investigations.[10]

• Clinical trials are human research studies that are used to assess a medicinal, surgical, or behavioral intervention. They are the most common technique for researchers to determine whether a novel treatment, such as a new medicine, diet, or medical equipment (such as a pacemaker), is safe and effective in humans. A clinical trial is frequently performed to determine whether a new treatment is more successful than the current treatment and/or has fewer negative side effects.[10]

• Clinical studies are often carried out in stages that build on one other. Each phase is intended to provide answers to specific questions. Knowing the clinical trial's stage is crucial, since it might give you a sense of how much is known about the medicine being investigated. [12]

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PHASE 0 CLINICAL TRIALS -Despite the fact that phase 0 studies are conducted in humans, they are not the same as the other stages of clinical trials. The goal of this phase is to assist the drug approval process go more quickly and smoothly. Researchers may use phase 0 trials to see if the medications do what they're supposed to do. This could save time and money that would otherwise be spent on later-phase experiments. .Phase 0 studies use only a few small doses of a new drug in a few people known as micro dosing study [11]

PHASE I CLINICAL TRIALS -Phase I studies of a new drug are usually the first that involve people. Phase I studies are done to find the highest dose of the new treatment that can be given safely without causing severe side effects. Study participants ranges from 20-100 healthy volunteers. .It determine safety and dosage.[13]

PHASE II CLINICAL TRIALS: A phase II clinical study is conducted if a new medication is found to be safe in phase I clinical trials and to see if it works in specific forms of cancer. The advantage that doctors seek is determined by the treatment's purpose. It could indicate that the cancer is shrinking or disappearing. Study participants are up to several 100 people with disease and length of study is up to several months to 2 years .Determine efficacy and side effects [14]

PHASE III CLINICAL TRIALS: Before being approved for general use, treatments that have been shown to work in phase II clinical trials must pass a third phase. Phase III clinical studies assess the novel treatment's safety and effectiveness against the current standard of care. Study participants are up to 300-3000 volunteers who have disease and length of study is 1 to 4 years.[14]

PHASE IV CLINICAL TRIALS: In phase IV studies, drugs that have been approved by the FDA are generally monitored for a long time. Even after thousands of patients have been exposed to a new therapy, not all of the treatment's side effects may be known.it is the practice of monitoring safety of drug after it has been released in the market[15]

V. INVESTIGATIONAL NEW DRUG (IND) APPLICATION

The filing of an Investigational New Drug (IND) application is the initial stage in the drug review process. The application to the US Food and Drug Administration (US FDA) for an exemption to send the product to investigators throughout the state has been submitted. To qualify for this exemption, the company must submit the required information via the IND. INDs are divided into two categories:

1. Commercial - For companies looking to have a new medicine approved for marketing.
2. Non-commercial (research) - for companies submitting Investigator IND, Emergency Use, and Treatment INDs. [17]

VI. NEW DRUG APPLICATION (NDA)

The NDA application is the formal proposal to the FDA by drug sponsors, like as biotech and pharmaceutical corporations, to authorize a new pharmaceutical for sale and marketing. Since 1983, every new drug or therapy has required approval of a New Drug Application (NDA) before being commercialized in the United States.[18]

The NDA documentation is expected to detail the drug's entire history, such as what happened during clinical trials, what the medicine's ingredients are, the conclusions of animal research, how the drug acts in the body, and how it is produced, processed, and packed.[17]

Once the FDA has reviewed the NDA, it issues one of the below mentioned three action letters:

- Approval Letter – This letter confirms that the drug has been approved.
- Approvable Letter – This letter shows that the drug will be authorized eventually, but that it will need to be corrected due to a few flaws such as labelling revisions.
- Not Approvable Letter - Indicates that the medicine will not be approved and provides a list of reasons why. [17]

Objectives of NDA

- Whether the medicine's proposed labelling (package insert) is acceptable and what it should include
- Whether the drug is safe and effective in its proposed usage, and whether the drug's advantages exceed the hazards
- Whether the production procedures and quality control measures employed to maintain the drug's identity, strength, quality, and purity are sufficient to maintain the drug's identity, strength, quality, and purity. [16]

VII. TIME REQUIRED FOR DEVELOPING NEW DRUG

Drug development takes a long time since a candidate drug is reviewed by regulatory authorities in numerous countries at every step of development before being released on the market. A new drug can take anywhere from 12 to 15 years to create, according to PhRMA (Pharmaceutical Research and Manufacturers of America, a pharma industry trade organization in the Americas). Preclinical testing takes roughly six and a half years. Phase-I trials last around 1.5 years; Phase-2 trials last about 2 years; Phase-3 trials last about 3.5 years; and regulatory body assessment and approval takes about 1.5 years. Once the prospective drug has been approved for use as a medication. It may be subjected to additional Phase-IV trials to gather more safety and effectiveness information.(17)

VIII. DRUG DEVELOPMENT COST ACCORDING TO STUDIES

The cost of medication development has been estimated to be anywhere from $314 million and $2.8 billion, according to the report. Olivier Wouters, an assistant professor of Medicine Policy at the London School of Economics and Political Science, Martin McKee, a professor of European Public Health at the London School of Hygiene & Tropical Medicine, and Jeroen Luyten, an associate professor of the Faculty of Medicine at the Leuben Institute for Healthcare Policy's Department of Public Health and Primary Care, were the authors. (16)

Drug development costs for therapeutic domains with five or more medications ranges from $765.9 million for central nervous system treatments to $2.7716 billion for cancer and immunomodulating drugs, according to the median estimates.(16)

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A Review of the Preparation of Regulatory Dossiers in CTD Format and ECTD Submissions

Komal A. Virkar*, Trupti Ghige, Atish B. Velhal, Vivekkumar Redasani.
Department of Pharmacy, YSPM'S Yashoda Technical Campus, Satara.415003.

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ABSTRACT:
The information needed to prepare dossiers for multiple countries is discussed in this article, as well as the CTD format, which is projected to considerably decrease the time and resources needed by the industry to compile global registration applications and reports. The International Conference on Harmonization of the electronic common technical document (eCTD) aims to revolutionize the pharmaceutical submission procedure. In comparison to paper submissions volume, more than three-quarters of individuals with eCTD expertise were able to shorten their total time to approval, and more than 90% of this group was able to demonstrate cost savings.

KEYWORDS: Regulatory,CTD, eCTD, Dossier, ICH.

I. INTRODUCTION:

Regulatory Dossier

Dossier: In English, a dossier is a collection or file of materials about a specific subject, particularly one containing thorough information about a person or a topic. Any formulation is designed for human use, i.e., to alter or investigate physiological processes. [1]

"Pharmaceutical" refers to the use of systems or pathological conditions for the benefit of the recipient. "A product for human consumption." Critiquing and evaluating pharmaceutical dossiers is a process. Administrative, chemistry, preclinical, and clinical data are all included in this product. Information and authorization issued by a country's regulatory agencies to "Marketing approval or Registration" is a term used to describe the process of supporting a product's marketing or approval in a country." Product License" or "Marketing Authorization". [2]

A dossier is a file document that is submitted for drug product approval in several regulatory jurisdictions based on their requirements. CTD is a harmonized format (template) for presenting data in the ICH regions, and it is submitted in many ways such as CTD, and e-CTD.

A dossier is a collection of documents that provide in-depth information about a specific person or subject. (Or) a collection of papers relating to a subject or a person. (Or) A dossier is a file document that contains detailed information about a drug product and is submitted to the regulatory authorities. [3]

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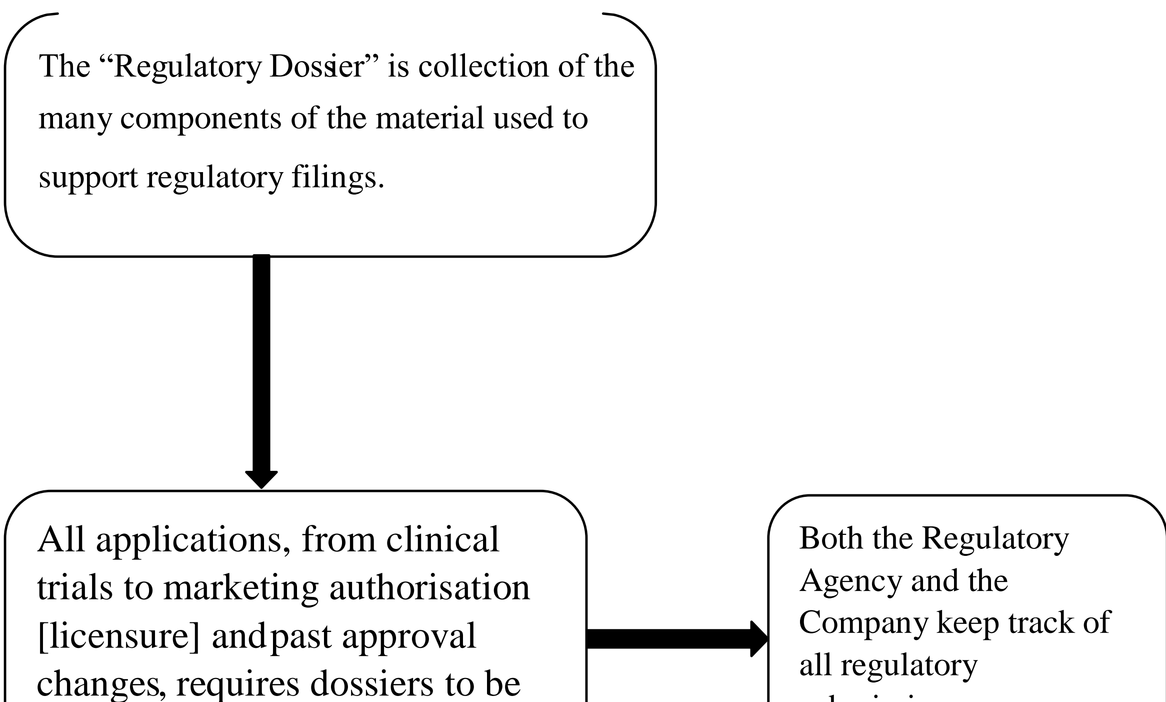

Figure 1: Regulatory Dossier Preparation.

COMMON TECHNICAL DOCUMENT (CTD):

A Common Technical Document (CTD) is a supporting list of leaflets that must be given to the regulatory body with pharmaceutical registration applications to obtain market authorization. CTD mostly describes the data format. It is customary for RA professionals to be aware of the documentation that must be provided when a medication product is approved. CTD, on the other hand, is primarily concerned with the orderly structure of information. CTD documents should be simple, straightforward, and transparent. [4]

CTD is an ICH-defined format that has been agreed upon and accepted by regulatory agencies in Europe, Japan, and the United States. The FDA defines the CTD as an information package containing clinical, non-clinical, manufacturing, and technical data that would be submitted for registration of novel pharmaceuticals in all three ICH regions, namely the United States, the European Union, and Japan. [8] Paper submission of ACTD and CTD format dossiers, as well as electronic submission of CD format dossiers, are used in semi-regulated markets such as ASEAN countries (Circle disk). [6]

(See Fig. 2 for a diagram of the CTD triangle describing the various modules.) As a result, has it five modules. [4]

1. Administrative and prescribing information (Module 1).
2. Common Technical Document (Module 2) Summaries (Quality Overall summary)
3. Module 3: Data of High Quality
4. Non-clinical study reports (Module 4)
5. Clinical Study Reports (Module 5) [13]

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CTD STRUCTURE:

Not part of the CTD

MODULE1
Regional
administration
information

MODULE 2
Quality overall summary
Non Clinical Overview
Clinical Overview
Non Clinical Summary
Clinical Study

The CTD

MODULE 3
Quality
MODULE 4
Non Clinical Study Report [Safety]
MODULE 5
Clinical Study Report [Efficacy]

Raw Data

Figure 2: Triagonal Representation of Dossier Preparation

Modules are divided into two categories:
First Regional module
Only the content of the shared modules is defined by the CTD. Each of the ICH regions defines the contents of Regional module1. (USA, Europe, Japan). [14]

ORGANISATION OF CTD:
The Common Technical Document is organized into five modules.

Module 1: Administrative Data.
Administrative information should include papers particular to each region, such as application forms or the proposed regional designation. [5]

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Module 2: Overall Quality Summary
CTD Synopsis Begin with a general overview of the drug (pharmacological class, mechanism of action, and intended clinical usage).
It begins with a general overview of the medicine, including its pharmacological class, mechanism of action, and potential clinical applications. Information (for example, pharmaceutical documentation), as well as the Non-Clinical and Clinical Overviews, NonClinical Written Summaries and Tabulated Summaries, and the Clinical Summary. [7]
Module 2 is divided into seven sections, which should be kept in the following order:
2.1Table of contents. 2.2 The Beginning
2.3 Overall Quality Summary. 2.4 Overview of Non-Clinical Research
2.5 Overview of Clinical Practice. 2.6 Non-clinical Summaries (Written and Tabulated)
2.7 Clinical synopsis
Module 3: Quality Assurance
The M4Q's Quality component establishes a standardized structure and method for delivering CMC (Chemistry, Manufacturing, and Controls) data in a registration dossier. The following are the primary headings in this section (which must not be changed): [5]
3.1 Module 3 Table of Contents
3.2 The data set
Drug Substance 3.2.S
Drug Product 3.2.P
3.3 Module 3 literature references. [11]

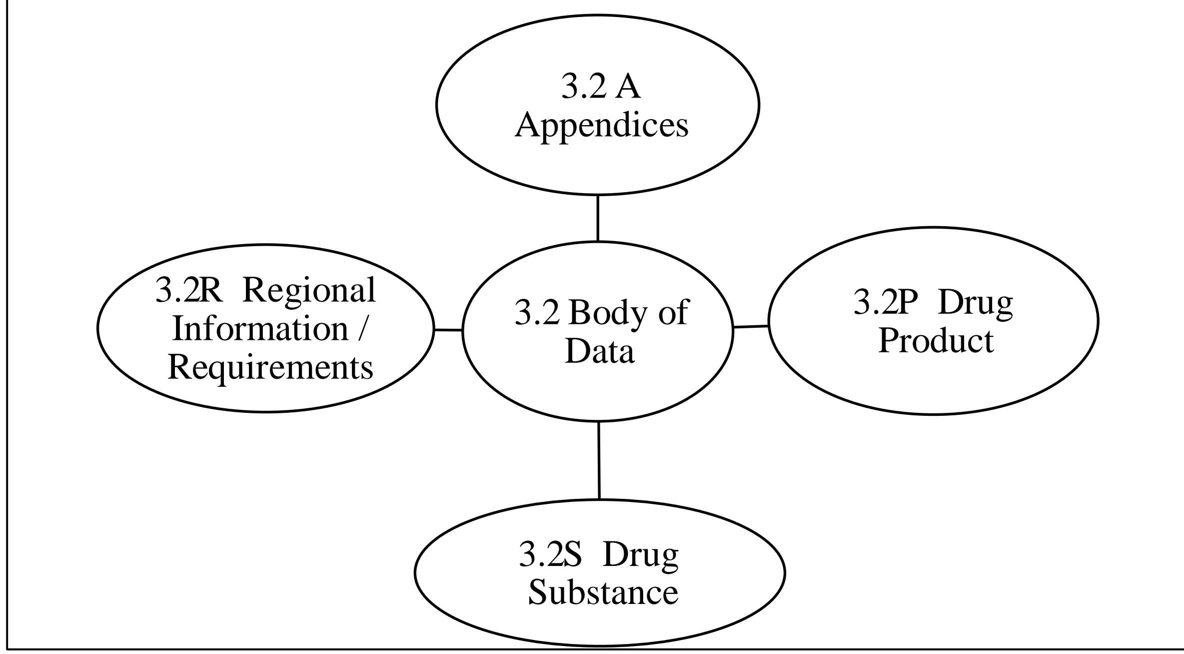

Figure 3: Dossier preparation in the CTD format.

Module 4: Reports on nonclinical and preclinical research
The CTD Safety (M4S) Guideline defines the nonclinical study's structure and format. Module 2 of the Common Technical Document summarises the information in Module 3 of the Common Technical Document and organizes Module 4 of the Nonclinical Study Reports.[16] The Nonclinical Overview should be no more than 30 pages long and should provide an integrated and critical assessment of the pharmaceutical's pharmacologic, pharmacokinetic, and toxicological examination. Nonclinical Written Summaries (100–150 pages) are indicated for more comprehensive summaries and discussions of nonclinical pharmacology,
pharmacokinetics, and toxicological information. [6]
4.1 Module 4 Table of Contents
4.2 Reports on research Pharmacology (section 4.2.1) [11]
4.2.2 Pharmacokinetics is a term that refers to the study of how drug toxicology (section 4.2.3)

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Module 4 uses 4.3 literature references. [5]

Module 5: Clinical Study Reports

The organization and format of clinical data in an application, including summaries and comprehensive study reports, is described by CTD-Efficacy (M4E). The Clinical Overview, a short document that gives a critical review of the clinical data, and the Clinical Summary, a larger document that focuses on data summary and integration, are both included in Module 2 of the CTD. Module 5 contains clinical study reports as well as raw data. [11]

The following are the primary headings in this section (which must not be changed):
5.1 Module 5 Table of Contents
5.2 A list of all clinical studies in a tabular format
5.3 Reports on clinical trials
5.3.1 Biopharmaceutical study reports
5.3.2 Reports on experiments involving human biomaterials and pharmacokinetics.
5.3.3 Human pharmacokinetic (PK) studies reports
5.3.4 Human pharmacodynamics reports (PD) research
5.3.5 Efficacy and safety study reports
5.3.6 Post-marketing experience reports
5.3.7 Individual patient listings and case report forms
5.4 Literature citations. [5]

ADVANTAGES OF CTD:

1. The main goal of implementing a common submission format is to make reviewing each application easier and to avoid other critical data or analysis missions. Omissions of this information can cause approvals to be delayed unnecessarily.
2. A common format for technical documentation will significantly reduce the time and resources required to compile applications for human pharmaceutical registration, as well as make electronic submission preparation easier. [6]
3. A standard document with common elements will be used to facilitate regulatory reviews and communication with the applicant.
4. The implementation of CTD is expected to significantly reduce the amount of time and resources required by the industry to compile global registration applications. [10]

CTD'S SILENT BENEFITS:

1. Global application harmonisation.
2. Establishes guidelines for preparing submission-ready documents during the IND stages.
3. Standardization makes project management and data management easier.
4. Makes life cycle management easier.
5. Aids in the planning of drug development. [10]

ELECTRONIC SUBMISSIONS (eCTD):

The electronic submission equivalent of the CTD is the eCTD. The eCTD serves as a conduit between industry and government agencies for the exchange of regulatory data, facilitating the development, review, lifecycle management, and archiving of electronic submissions. All CTD information is included in the eCTD submissions. The structure of the submission is represented by an XML file (Extensible Mark-up Language) at the heart of eCTD. It contains links to files as well as other metadata such as checksum data. The XML scheme is extremely rigorous. CTD submission, all subsequent submissions for the application should be in eCTD format. The submission's lifecycle management is simplified using eCTD. [7]
The electronic Common Technical Document (eCTD) is a regulatory information transfer link between the pharmaceutical industry and regulatory agencies. The Common Technical Document (CTD) format is used for the main content. The Multidisciplinary Group 2 Expert Working Group (ICH M2 EWG) of the International Conference on Harmonisation (ICH) produced it. [15] Essentially, the electronic Common Technical Document (eCTD) will be a transport format that will allow electronic submissions to be moved into an agency's review environment. The eCTD will act as an interface for the flow of regulatory information from industry to agencies, while also making the production, evaluation, lifecycle management, and archiving of electronic submissions easier. [17]
An eCTD application is a CTD application, but then electronically.

Electronically means for eCTD:

I complete the dossier in electronic format I XML files (XML backbone)
I Specifications followed for the Granularity, folder- & filename convention of the dossier Navigation through the dossier using hyperlinks and bookmarks. [9]

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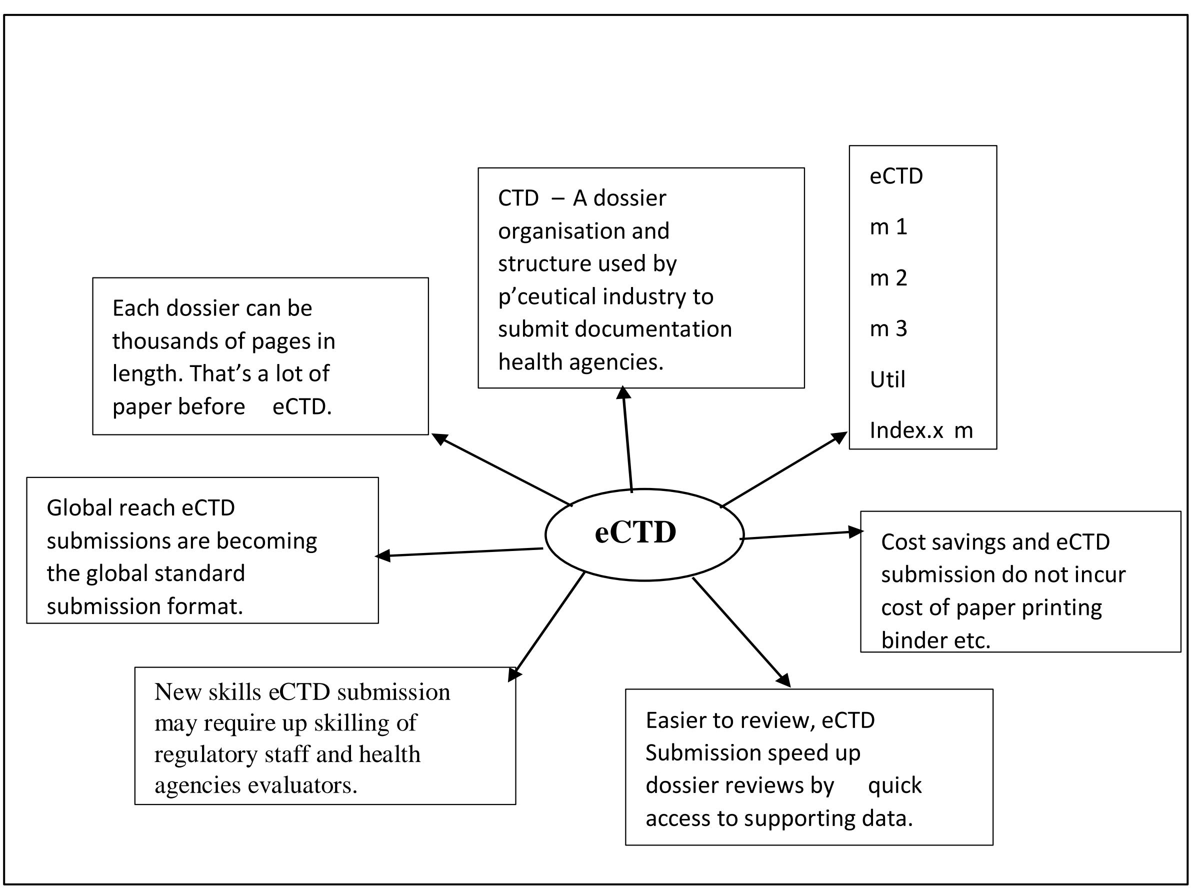

Figure 4: Overview of eCTD Submission.

eCTD Submission Checklist:
eCTD Software
Software training and support from the supplier
Compiling and eCTD

eCTD hyperlinking
QC of eCTD

Submit eCTD on CD/DVD or Use an electronic gateway [2] **eCTD STRUCTURE:**
eCTD is highly recommended by USFDA for NDAS, BLAS, DMFS, and INDs filing. From the year 2010 European Union also make compulsory for electronic CTD submission to all procedures. [2]

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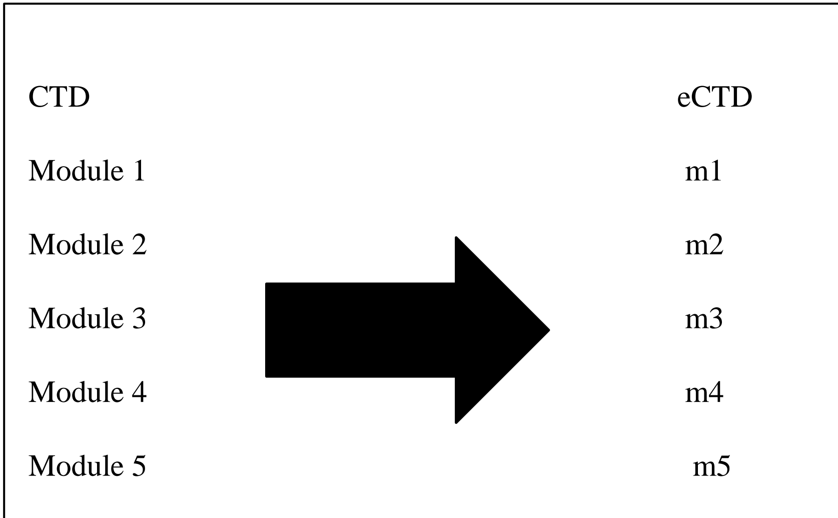

Figure 5: Electronic Submission to all Procedures.

eCTD ADVANTAGES:

The eCTD dossier becomes the single authoritative regulatory archive, thus reducing the use and costs associated with producing and storing paper dossiers. Enhanced ability to organize, prepare, and manage submission content.

Opportunity for streamlined interactions with agency reviewers, decreased response times to agency requests, and ultimately, a faster approval timeline.

Facilitates collaboration between teams of document authors, reviewers, publishers, and external partners. [6]

There are five modules in eCTD as mentioned here:

1. Region-specific information.
2. Summary documents.
3. Information related to quality.
4. Non-clinical study reports.
5. Clinical study reports (CSRs).

Comparison OF CTD and eCTD:

Sr. No.	Paper CTD [Common Technical Document]	eCTD [Electronic Common Technical Document]
1.	Volumes, tabs, and slip sheets were entered electronically and then printed on paper.	Electronically filed with e-documents in folders.
2.	A4 paper must be used.	A4 or US letter size documents are acceptable.
3.	TOCs and volume are used to navigate the CTD.	XML backbone for eCTD navigation.

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4.	The target CTD section number is included in the cross-reference.	The target is linked to the cross-reference.
5.	TOCs, page numbers, and caption crossreferences are used to navigate the document manually.	TOCs, bookmarks, and hyperlinks are used to navigate electronic documents.
6.	Trucks delivered binders in boxes on pallets.	CD (or DVD) or email portal submissions are accepted.

Table 1.CTD and eCTD Statements in Comparison.

eCTD submissions are accepted for the following applications:

1. Investigational New Drugs (INDs).
2. New Drug Applications (NDAs).
3. Abbreviated New Drug Applications (ANDAs).
4. Biologics License Applications (BLAs).
5. All the applications following submission of the above-stated applications.
6. All the Master Files (MFs) are part of any above-mentioned applications.

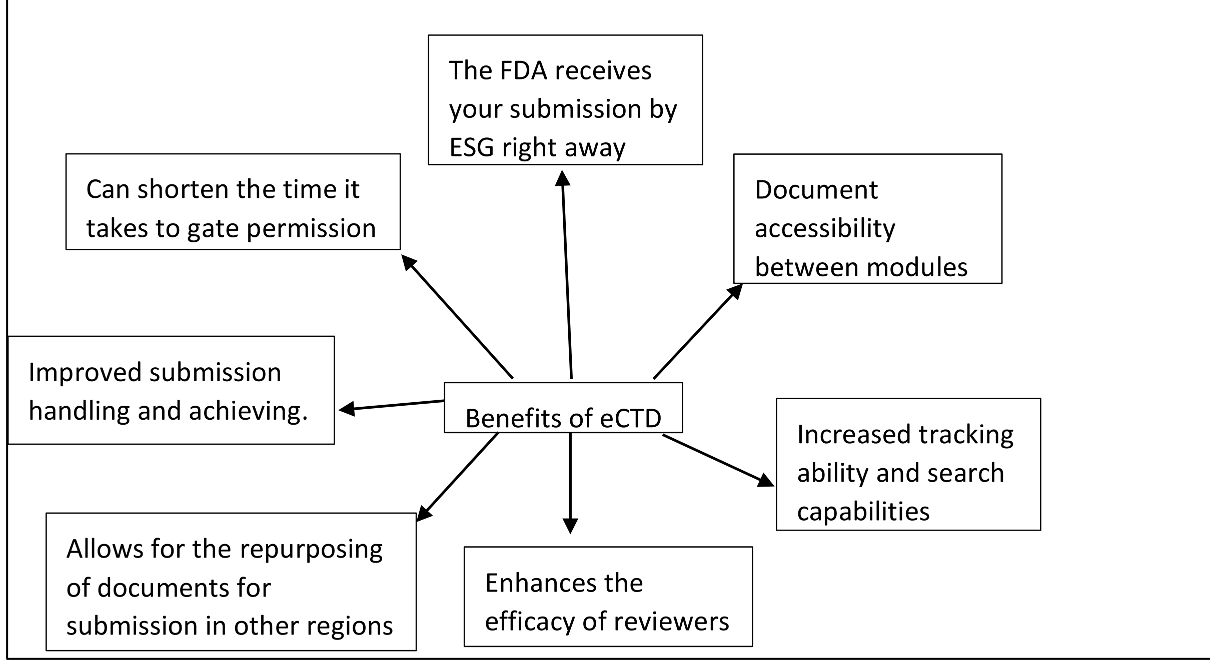

Figure 5: Overview of Benefits of eCTD

Content specification– as defined by ICH specified below-

1. Technical specification- Electronic software
2. CTD -TOC [pdf] [paper]
3. eCTD- XML Backbone

The eCTD is an electronic document similar to the CTD. Is an eCTD backbone describing the Structure of the submission, the XML file (Extensible Mark-up Language) includes links

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to files and other metadata such as checksum information.

1. The schema for the XML is very rigid.
2. Easy to distribute and review.
3. More efficient use of resources with less cost and stress to the organization.
4. Self-validating.

The eCTD Requirements:

You must submit electronic submission using the FDA's current supported version of eCTD.
The current version of eCTD, that is supported is listed in the Data standards Catalog and further explained in the technical specification document below.
Electronic Common Technical Document Specification of the International Council for Harmonisation (ICH). Study tagging files ICH eCTD Backbone file specification. [15] **Software used in eCTD management:**[9]

Sr. No	Software
1.	ECTDXPress-Image solution-http://www.imagesolutions.com.
2.	ECTDXPress-Image solution-http://www.imagesolutions.com.
3.	Data farm, http://www.datafarminc.com.
4.	Take solution: www.PharmaReady.com.
5.	MasterControlsubmissionGateway™-MasterControl, http://www.mastersolution.com.
6.	Lorenz Life Science: www.lorenz.com.

Table 2. Software used in eCTD Submission.

Things to know before using eCTD software
eCTD knowledge
General eCTD tool knowledge
Document format types and editing
Software functions and system requirements
FDA ESG submission requirements
FDA Guidance Submission format delivery

How to send it to Authorities?
Files on a CD or DVD
Files attached to an e-mail
Files submitted via Eudralink
Files submitted via CESP 1 File submitted via a Gateway. [2]

II. CONCLUSION:

Any export market requires a high-quality dossier, which may be created via a methodical Formulation Development process. The right planning and execution of Formulation development will aid in the production of high-quality dossiers and the response to regulatory bodies' questions. It is critical to assemble documentation in a format that is acceptable internationally for both regulated and non-regulated markets when registering pharmaceutical products in any of the exporting countries. Due to significant discrepancies in the requirements for dossier registration for pharmaceutical products, the CTD and eCTD formats were developed. This aids in the compilation of documents in the above-mentioned format as per the registering requirements.
According to the thesis, the way of submitting a Dossier, according to CTD and eCTD format, Module 1- contains Administrative Information, Module 2- contains the Overall summary, and Module 3- contains the Quality Information. In

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summary, Module 4 contains preclinical data, while Module 5 contains clinical data.

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Comprehensive Review On Gmp Of Pharmaceutical Products

UTKARSH M. PATIL, MR. A. B. VELHAL, DR. V. K. REDASANI, ANAGHA A. BAGAL
STUDENT, PROFESSOR, PRINCIPAL, STUDENT
DEPARTMENT OF PHARMACY,
YSPM'S YASHODA TECHNICAL CAMPUS, SATARA, INDIA

Abstract: The concepts of GMP are not new, it is from ancient times. The concept required for GMP are explained in this report. The main purpose of GMP is preventing mistakes and errors involved in any manufacturing activities. To achieve agreement of guidelines and laws of the manufacturing of medical products for human use there are some public and also private organizations institutes and regulatory authorities who work and cooperate with pharmaceutical industry.
GMP guidelines provide minimum requirements for pharmaceutical or a food product manufacturer must meet to assure that the products are of high quality and do not pose any risk to the consumer or public. Good manufacturing practices (GMP) is a part of quality assurance which ensures that products are consistently produced and controlled to the quality standards appropriate to their intended use and as required by the marketing authorization.

***Index Terms* - GMP, Manufacturing activities, Guidelines, Quality assurance, Quality Standards.**

I. INTRODUCTION

The concept of Good Manufacturing Processes was introduced to regulate packaging and manufacturing processes in the pharmaceutical industrial areas.[1]
Manufactures follows various procedures and principles for the therapeutic good which helps in ensures the required quality of products. Good manufacturing practices is the component of quality assurance which helps in ensure the ensure the products are consistently manufactured and controlled to the Quality Standards appropriate to their intended use.
Good manufacturing practices is mainly used to reduce risk involved in production of pharmaceuticals products that cannot be removed through testing of the final products. Good Manufacturing practices covers all views of production from initiating materials, equipment's, premises to personal hygiene and training of employees.
A basic principle of Good Manufacturing Practices is that quality cannot be tested into a batch of product but must be built into each batch of product during all stages of the manufacturing process. It is designed to minimize risk involved in any pharmaceutical production that cannot be eliminated through testing the final product.

Good Manufacturing Practices:
The quality of formulation and bulk drug depends on the quality of those producing it.
Good manufacturing practices is the magic key that opens the door quality
In matter of GMP swim with the current and in matter of quality stand like a rock
Most countries will only accept import and sale of medicine that have been manufactured to internationally recognized GMP
Government Seeking to promote their countries export of pharmaceuticals can do so by making GMP mandatory for all pharmaceutical production and by training their inspectors in GMP requirements. [2,3]

Why GMP is important:

- A poor-quality medicine may contain toxic substance that has been unintentionally added.
- A medicine that contains little or none of the claimed ingredients will not have the intended therapeutic effect. Provides a high-level assurance that medicines are manufactured in a way that ensures their safety efficiency and quality.
- It maintains the consistency in the manufacturing of the medical products.
- To eliminate contamination and to minimise the error GMP is important.

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➢ Good Manufacturing Practices ensures companies execute consistent procedures in safe environments.

➢ Good Manufacturing Practices helps in ensure the proper design, monitoring and control of manufacturing process and facilities, while securing the identity, strength, quality of their products.

➢ Good Manufacturing Practices assist cutdown on facility losses and waste and also to protect consumers the manufacturer from harm. [3,4]

How to Comply with Guidelines:
GMP guidelines and regulations address different issues that can influence the safety and quality of a product. Meeting GMP or cGMP standards helps the organization comply with legislative orders, increase the quality of their products, improve customer satisfaction, increase sales, and earn a profitable return of investment. [5,6]
Conducting GMP audits play a big part in assessing the compliance of the organization to manufacturing protocols and guidelines. Performing regular checks can minimize the risk of adulteration and misbrand. A GMP audit helps improve the overall performance of different systems including the following:

- Building and facilities
- Materials management
- Quality control systems
- Manufacturing
- Packaging and identification labelling
- Quality management systems
- Personnel and GMP training
- Purchasing
- Customer service

Good manufacturing practice - the general/current state:

➢ **Pharmaceutical quality system:**

This guideline describes a comprehensive model for an effectiveness quality system of medicinal products, based on the concepts of ISO quality and its implementation throughout all stages of the lifecycle of the product.
The guideline applies to supporting the development and manufacture of substances of Pharmaceutical Industry, Active Pharmaceutical Ingredient and medicinal products, including biotechnology and biological products throughout the life cycle of the product.
Quality assurance is a broad concept that includes all matters that individually or collectively influence the quality of a product, that is, management of the quality of raw materials, products and other components, services related to production, and management, production and inspection processes. It is applied in pre-production to verify what will be made meets specifications and requirements and also while manufacturing production. [7,8]
Personnel:
According to GMP, the management of an enterprise should determine and provide appropriate resources such as human resources, financial, materials, facilities and equipment to implement and maintain the Quality Management System and improve effectiveness. Effective coordination and management of human resources are key factors in the proper functioning of any enterprise system and improve effectiveness.
For the maintenance of satisfactory system of quality assurance and the correct manufacture and control pharmaceutical products there must be sufficient qualified personnel to carry out all the tasks for which manufacturer is responsible.
Personnel should be aware of principles of GPM that affects them and receive initial and continuing training, including hygiene instructions, relevant to their need. [9,10]
Premises and equipment:
Premises and equipment must meet and comply with all rules, according to the operations to be performed in order to minimize the risk of errors and should allow effective cleaning and maintenance. [11,12]
Some examples are:
a. Walls: Walls in manufacturing areas, packaging areas and corridors should be of plaster finish on high-quality concrete blocks or gypsum board. The finish should be smooth, usually with enamel or epoxy paint. They should be washable and able to resist repeated applications of cleaning and disinfecting agents.
b. Floors: Floor covering should be selected for durability as well as for clean ability and resistance to the chemicals with which it is likely to come into contact. Epoxy flooring provides a durable and readily cleanable surface.
c. Ceilings: Manufacturing areas require a smooth finish, often of seamless plaster or gypsum board. All ceiling fixtures such as light fittings, air outlets and returns should be designed to assure ease of cleaning and to minimize the potential for accumulation of dust.

Raw Material:
All materials used for production should be stored properly according to the appropriate conditions which are set by the manufacturers.
There should be a proper stock management system implemented to ensure that all incoming materials are correct and of high quality. [13]

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Documentation:
Good documentation constitutes an essential part of the quality assurance system and it is the key to operate in compliance with GMP requirements.
All types of documents and media used should be fully defined in the manufacturer's Quality Management System.
Given below is a list of the most common types of documents along with a brief description of each [14,15]
Site Master File: A document describing the GMP related activities of the manufacturer.
Quality Manual: A global company document that describes, in paragraph form, the regulations and/or parts of the regulations that the company is required to follow.
Policies: Documents that describe in general terms, and not with step-by-step instructions, how specific GMP aspects (such as security, documentation, health, and responsibilities) will be implemented.
Logbooks: Logbooks are used for documenting the operation, maintenance, and calibration of a piece of equipment.
Logbooks are also used to record critical activities, e.g., monitoring of clean rooms, solution preparation, recording of deviation, change controls and its corrective action assignment.
Test Methods: These documents are typically used and completed by the quality control (QC) department. Test methods provide step-by-step instructions for testing supplies, materials, products, and other production-related tasks and activities, e.g., environmental monitoring of the GMP facility.[16]

Production:
Production operations must clearly follow the procedures. They must comply with the principles of GM Pin order to obtain quality products and be in accordance with the relevant manufacturing.
All handling of materials and products, such as reception and quarantine, sampling, storage, labelling, dispensing, processing, packaging and distribution should be done in accordance with written procedures or instructions and where necessary, recorded.[17]

Quality control:
Quality control is concerned with sampling, specifications and testing as well as the organization, documentation and release procedures which ensure that the required and relevant tests are carried out, and that materials are not released for use, nor products released for sale or supply, until their quality has been judged satisfactory.
QC is not confined to laboratory operations, but may be involved in many decisions concerning the quality of the product.[18]

Quality risk management
Quality risk management is a systematic process of assessing risks that can affect the quality of the product. According to its principles, quality risk management should ensure that:
The evaluation of the risk to quality is based on scientific knowledge, experience with the process and ultimately links to the protection of the patient and users;
The level of effort, formality, and documentation of the quality risk management process is commensurate with the level of risk.
c) The general quality risk management process and integration into the product quality can be referred to in ICHQ9. [19,20]
Validation and qualification
Qualify systems, premises, and equipment if they are fit/ready for their intended use and validate if processes and procedures can repeatedly produce high-quality products. Critical steps in the manufacturing process should be verified to ensure that product quality is consistent and maintained at a high level. According to the WHO (World Health Organization), qualification and validation should establish and provide documentation stating that:
the premises, supporting utilities, equipment, and processes have been designed in accordance with the requirements for GMP (design qualification or DQ)
the premises, supporting utilities, and equipment have been built and installed in compliance with their design specifications (installation qualification or IQ);
the premises, supporting utilities, and equipment operate in accordance with their design specifications (operational qualification or OQ); and a specific process will consistently produce a product meeting its predetermined specifications and quality attributes (process validation or PV, also called performance qualification or PQ) [21,22.23]

Self-inspection:
The objectives of self-inspections are the evaluation and supervision of compliance of the manufacturer with GMP in all aspects of production and quality control. It must be designed to detect any deficiency in the implementation of GMP and to recommend corrective procedures.[24]
Sanitation And Hygiene: -
A high level of sanitation and hygiene should be practiced in every aspect of the manufacture of medicine products. The scope of sanitation and hygiene covers personnel, premises, equipment and apparatus, production materials and containers, products for cleaning and disinfection, and anything that could become a source of contamination to the product. Potential sources of contamination should be eliminated through an integrated comprehensive programmed of sanitation and hygiene
The areas, surfaces, and equipment in and on which products are made must be kept clean. Dirt, and the microbes that it can harbor, must not get into or on products. Disinfectants can be inactivated by dirt. Dirt (particularly oily or greasy films and protein like matter) can also protect microorganisms against the action of disinfectants. So, before disinfection, it is important to first clean surfaces. Where gross amounts of dirt are present, it may be necessary to first remove most of it by scrubbing. Then surfaces may be cleaned by the application of a cleaning agent, followed by rinsing. [24,25,26]

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Basic requirements for active substances used as starting materials:
This guideline is intended to provide guidance regarding GMP for the manufacture of active substances under an appropriate system for managing quality. It is also intended to ensure that active substances meet the requirements for quality and purity that they purport or are represented to possess.
These guidelines apply to the manufacture of active substances for medicinal products for human use and to the manufacture of sterile active substances only up to the point immediately prior to the active substance being rendered sterile.[27]
Manufacture of medicinal products-
Manufacture of solid and semi-solid medicinal product:
Since this type of medicinal products is particularly susceptible to microbial contaminants and other contaminants during manufacturing, it is necessary to follow preventive procedures and it should be apriority for the manufacturer MA holder.[28]
Manufacture of herbal medicinal product:
The procedures and techniques used in the manufacture and quality control of herbal medicines are often substantially different from those used for conventional medicinal products. The herbal substance should be of suitable quality. The supporting data should be provided to the manufacturer of the herbal medicinal products. These guidelines apply to all herbal starting materials: Medicinal plants, herbal substances or herbal preparations. These guidelines apply to all herbal starting materials: Medicinal plants, herbal substances or herbal preparations.[29]

Manufacture of biological active substances and medicinal products for human use:
The methods employed in the manufacture of biological active substances and biological medicinal products for human use are critical factors in shaping the appropriate regulatory control, because the manufacture of these involves certain specific, considerations arising from the nature of products and manufacturing processes, being necessary take some special precautions. Unlike conventional medicinal products, which are normally produced and controlled using reproducible chemical and physical techniques, biological products are manufactured through methods that involve biological processes and materials, such as cultivation cells or extraction of material from living organisms.[30]

Manufacture of sterile medicinal product:
The manufacture of sterile products requires special requirements in order to minimize risks of microbiological contamination, and of particulate and pyrogen contamination, being highly dependent on knowledge, training and attitudes of the personnel involved. This type of manufacture must strictly follow methods and preparation processes, carefully established and validated, since the quality assurance, is of particular importance.[31]
sampling of starting and packaging material:
Sampling is an operation where a small fraction of the batch is removed integrating operations to select a portion of a pharmaceutical product for a specific purpose, in accordance with an appropriate procedure. This process should be carried out in accordance with written and approved procedures that are appropriate to the sample and the type of control intended to be applied to the sample and the sample material.[32]

II. Acknowledgment

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Drug Regulatory Affairs - Role of Regulatory Affairs in the Pharmaceutical Industry

Akash Hitnalli*, Ganesh Devane, Atish Velhal

Department of pharmacy, Yashoda Technical Campus, Satara, 415003. India.

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ABSTRACT

Pharmaceutical drug regulatory affairs govern the registration parameters of pharmaceutical products. It has an extensive spectrum protecting all factors of documentation and advertising and marketing in legalized form. The pharmaceutical enterprise is a distinctly regulated industry in our country. Regulatory affairs gurus want the current market state of affairs to cater to hyperlink pharmaceutical industries and global regulatory agencies. Regulatory affairs (RA), is an occupation inside synchronized several industries, such as pharmaceuticals, clinical gadgets, and biotechnological industries. Regulatory Affairs additionally has a very precise which means inside the pharmaceutical industries. DRA is a dynamic, moneymaking subject that consists of each scientific and felony element of drug development. Regulatory affairs gurus assist the organization keeps away from issues precipitated using badly stored records, inappropriate scientific wondering, or bad presentation of data. In most product areas the place regulatory necessities are imposed, and restrictions are additionally positioned upon the claims which can be made for the product on labeling or in advertising.

INTRODUCTION

Regulatory Affairs (RA), also called Government affairs is a profession within regulated industries such as pharmaceutical, medical devices, energy, & banking. Regulatory affairs also have a very specific meaning within the healthcare industries (medical devices, pharmaceuticals, biology's functional foods). Most companies, whether or not they are the most important multinational pharmaceutical firms or small, progressive biotechnology organizations have expert departments of Regulatory Affairs professionals [1]. Nowadays the pharmaceutical industry is well organized, systemic, and compliant with international regulatory standards for the manufacturing of chemical and biological drugs for human and veterinary consumption as well as medical devices, traditional cosmetics, and herbal products. Each regulatory system had faced certain circumstances which led to the current well-defined and controlled regulatory framework this has resulted in systemic manufacturing and marketing of safe, efficacious, and qualitative drugs. With the growth of industry, the legislations from each region have become more and more complex and created a need for regulatory professionals. [2]

The regulatory professional's job is to keep track of ever-changing legislation in all the regions in which the company wishes to distribute its products they also advise on the legal and scientific control and requirements. They are responsible for the presentation of registration documents to regulatory agencies and carry out all the subsequent negotiations necessary to obtain and maintain marketing Authorization for the product they give strategic technical advice at the highest level in their companies, right from the beginning of the development of a product, making an important contribution both commercially and scientifically to the success of a development program and the company as a whole. The demand for Regulatory Affairs (RA) professionals is evident across the pharmaceutical industry, consultancy companies, clinical research organizations, and regulatory agencies. [3,4]

History of Regulatory Affairs

Modern medicine regulation started in 19th-century life sciences, especially in pharmacology, chemistry, and physiology. Which laid a foundation for modern drug development and research and started to develop successfully after the Second World War. Unfortunate events have catalyzed the development of medicines regulation more than the evaluation of knowledge base. In 1937 over 100 people died of diethylene glycol poisoning following the use of a sulfonamides elixir in the United States which used the chemical as a solvent without

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any safety testing this facilitated introduction of the federal food. Drug and cosmetics act with premarket notification requirement for new drugs in 1938 However, in countries with a poor regulatory environment even recently medicines contaminated with diethylene glycol have killed patients.[5]

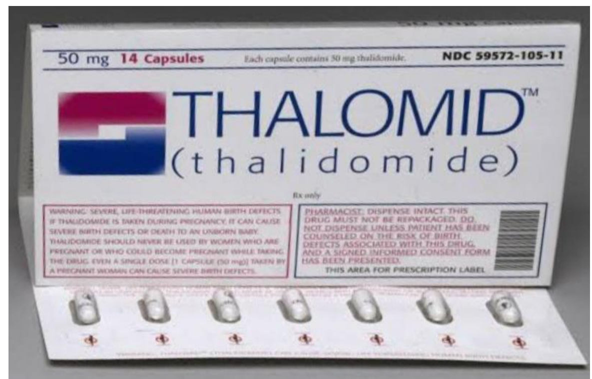

Figure No. 1: Thalidomide capsule.

The second incident that influenced the development of medicines regulation more than any event in history was the thalidomide disaster Thalidomide was a sedative and hypnotic that first went on sale in western Germany in 1956 between 1958 and 1960 it was introduced in 46 different countries. Worldwide resulting in an estimated 10,000 babies being born with phocomelia and other deformities. As result, the whole regulatory system was reshaped and substantially increased legislation for drug product quality, safety, and efficacy. This has resulted in stricter norms for Marketing Authorization (MA) and good manufacturing practices (GMPs).[6]

Role of Regulatory Affairs

Regulatory Affairs (RA), is professional within regulated industries Regulatory affairs also have a very specific meaning within the health care industries. The companies are responsible for the discovery, testing, manufacture, and marketing of these products and also want to ensure that they supply products that are safe and make Worth While contributing to public health and welfare. Regulatory Affairs is a professional developed from the desire of governments to protect public health by controlling the safety and efficacy of products in areas including pharmaceuticals, veterinary medicine, medical devices, pesticides, agrochemicals, and cosmetics by the companies. The main need of regulatory affairs is to provide the basis for the assurance of the high quality of food products which can increase Consumer's interest in ensuring efficacy, quality, and safety. [7]

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Regulatory Affairs (RA) professionals are employed in industry, government regulatory authorities, and academics. They aim to protect public health in terms of the safety, quality, and efficacy of products like medical devices, pharmaceuticals, veterinary medicines, pesticides, cosmetics, complementary medicine, etc. The wide range of regulatory professionals includes this area. pharmaceuticals, cosmetics, medical devices, biologics and biotechnology, and in-vitro diagnostics. Regulatory Affairs are vital to the proper functioning of society and economies. Regulation protects the rights, safety, and health of citizens and ensures the safe and effective delivery of public goods and services.[8]

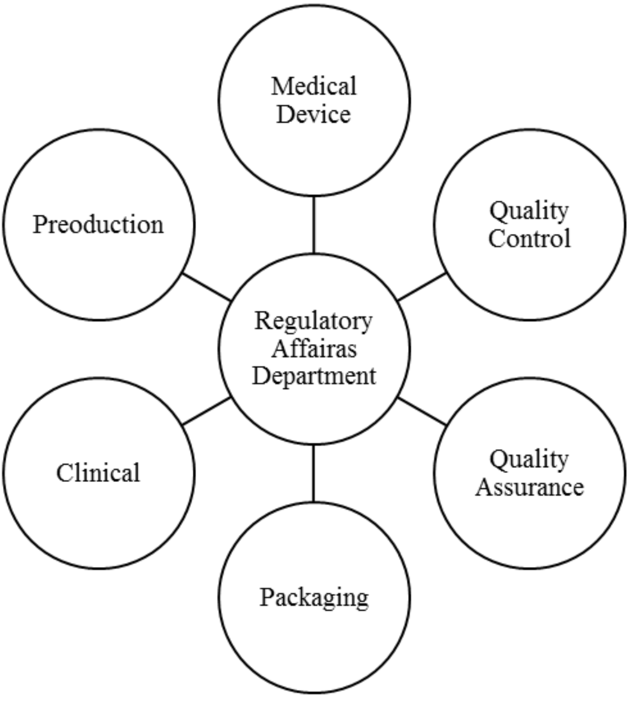

Figure No. 2: Role of Regulatory Affairs

In today's competitive environment the reduction of time taken to reach the market is critical to products and hence the Company's success. The proper conduct of its Regulatory activities is therefore of considerable economic importance for the company. A new drug might also have a value of many tens of millions of Euros or dollars, pounds, to enhance and even a three-month extend in bringing it to the market has sizeable monetary issues and even worse screw-ups to completely record all the on-hand facts or launch of the product being incorrect, labeling might also without problems result in the want for a product recall both incidences may additionally lead to the loss of various hundreds of thousands of units of sales. Not to mention the resulting reduction in confidence of the investors, health professionals, and

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patients. The regulatory affairs department is very often the first point of contact between the government authorities and the company. [9,10]

The key role of Regulatory Affairs (RA) professionals is broader than registration products. They advise the company both strategically and technically at the highest level. Their role begins right from the development of the product to make, marketing, and post-marketing strategies. Their advice at all stages both in term of legal and technical requirements help companies save a lot of time and money in developing the product and marketing the same for countries that do not have their regulations the world health organization guidelines on health matters and world trade organization on trade regulations between nations is followed. [11,12]

Regulatory Bodies in the World

Every country has its very own regulatory authority which is accountable to implement the regulations and policies and problem tips for drug development, licensing, registration, manufacturing, advertising and marketing, and labeling of pharmaceutical products. The regulatory bodies are given in table 1. [13,14]

Table No. 1: Regulatory Bodies in the World

Sr no.	Country Name	Regulatory Body
1	USA	Food And Drug Administration (FDA)
2	UK	Medicine and Healthcare Products Regulatory Agency (MHRA)
3	Australia	Therapeutic Goods Administration (TGA)
4	India	Central Drug Standard Control Organization (CDSCO)
5	Canada	Health Canada
6	Europe	European Medicines Agency (EMEA)
7	Japan	Ministry Of Health, Labor Welfare (MHLW)
8	Ukraine	Ministry Of Health
9	China	State Food And Drug Administration
10	Germany	Federal Institute For Drugs and Medical Device

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Pharmaceutical drug Regulatory Affairs

This branch is accountable for understanding the regulatory necessities for getting new products approved. They recognize what commitments the organization has made to the regulatory corporations in the place the product has been approved. They additionally post annual reviews and dietary supplements to the agencies. Regulatory Affairs usually communicates with one of the facilities at the FDA headquarters as an alternative to the FDA's nearby district offices. However, they need to recognize and consider adjustments to drug manufacturing and check out things to do to decide if and when the FDA have to be notified the organizations accountable for the discovery, testing, manufacture, and advertising of this merchandise additionally prefer to make certain that they grant merchandise that is protected and make a worthwhile. Contribution to public fitness and welfare. [15,16]

Drug Development Process and Clinical Trial

The innovator company synthesis a New Chemical Entity (NCE) or New Biological Entity (NBE) which can probably be a cure for a disease. The synthesis of NCE/NBE takes place in the preclinical testing period. The innovator company after the synthesis of an NCE/NBE files an Investigational New Drug (IND) application and requests the FDA to grant permission to conduct clinical trials. Clinical trials today have become one of the most important aspects of modern medical research and drug development.[17]

After studying the IND application, FDA grants permission to conduct clinical trials which involve studies in phases like phase1, phase 2, and phase 3. The innovator company files New Drug Application (NDA) and requests the FDA to grant permission to commercialize the product after studying the application, FDA grants permission to launch the new drug in the market the company continues clinical trials of the same molecule in phase 4 called product surveillance studies. Not every compound that is tested in the laboratory is marketed but before is marketed it has undergone several stages of development called drug development. The development of a new drug is a complex and costly process the cost for the development of biopharmaceuticals is higher than those specified earlier. About 10,000 NCE investigated to potentially treat disease, only 250 might make it to the animal testing and these approximately 5-10 would qualify for testing in humans, which means 1-2 of the original 10,000 NCE results in a marketable product. [18,19]

There are five important steps including many phases and stages each of them.

The five steps are:-

1) Step 1: Discovery and Development
2) Step 2: Preclinical Research
3) Step 3: Clinical Development
4) Step 4: FDA Review
5) Step 5: FDA post-market safety monitoring.

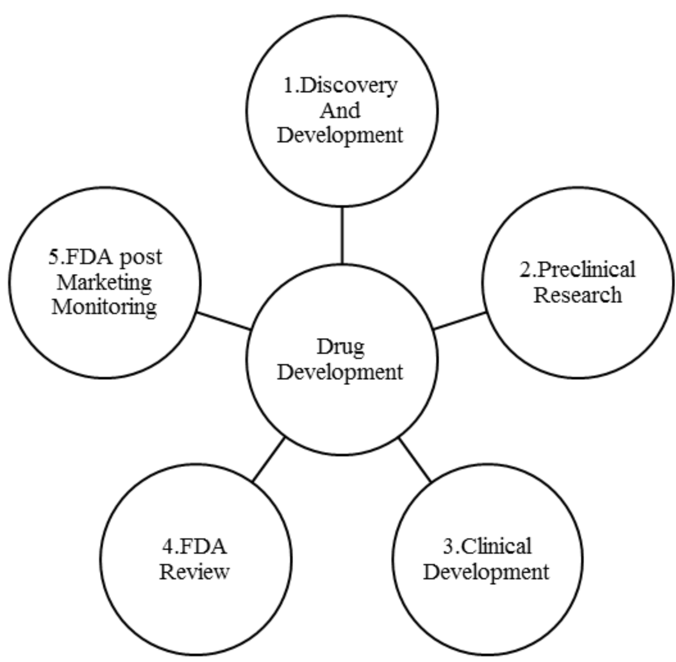

Figure No. 3: Drug Development Process

Step 1: Discovery and Development

Drug discovery efforts address a biological target that has shown to play a role in the development of the disease or starts from a molecule with interesting biological activities. The drug discovery process involves the identification of candidate drugs in their synthesis, characterization, screening, and assay for therapeutic efficacy.

Stages of Drug Discovery

1. Target Identification
2. Target Validation
3. Lead Identification
4. Lead Optimization
5. Pre-Clinical Safety
6. Clinical trials

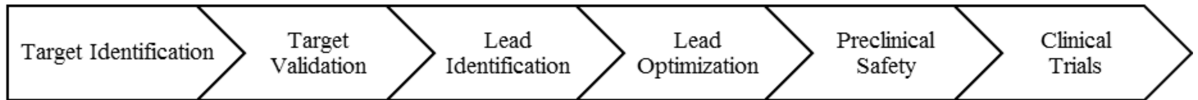

Figure No. 4: Stages of Drug Discovery

Step 2: Preclinical Research

The preclinical research step comprises studies on animals to find out various parameters for a potential drug candidate under the process of development during this stage a sponsor evaluates the drug's toxic and pharmacological effects through in-vitro and in-vivo laboratory animal testing. At the preclinical research step, the US FDA's minimum requirement is that a sponsor should develop a pharmacological profile of the drug; determine its acute toxicity in at least two species of animal and conduct short-term toxicity studies ranging from 2 weeks to 3 months, depending on the proposed duration of use of the candidate drug in the proposed clinical studies.[20]

Step 3: Clinical Development

The clinical development step involves the development of potential drug candidates comprised of pharmaceutical clinical trials which are commonly conducted in 4 phases.

Phase 0: This is an exploratory phase of a clinical trial that expedite the development of a promising drug by establishing early on whether the agent behaves in human subjects as anticipated from preclinical studies.

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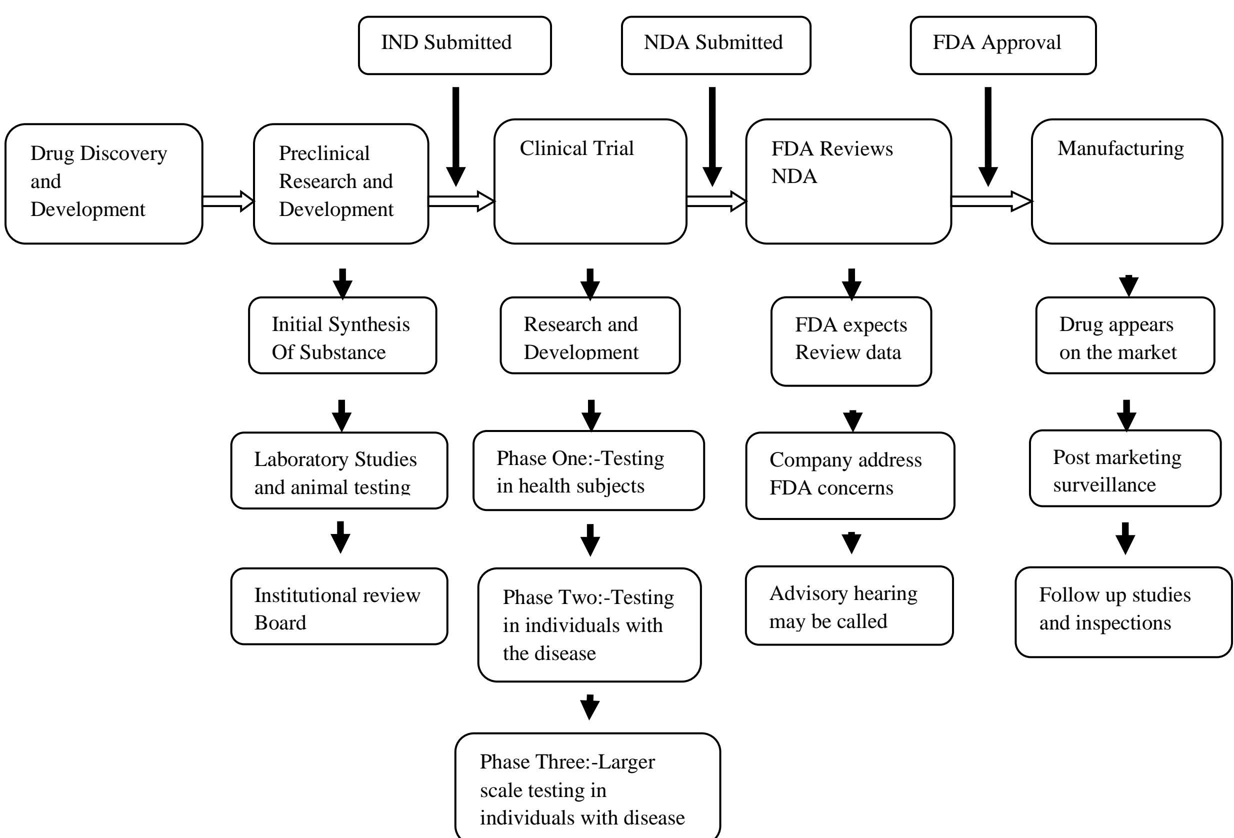
Phase 1: Studies in phase 1 are carried out on a small number of healthy volunteers usually 20 to 100 with the disease or condition and the study requires several months. The purpose of studies in this phase is to identify the metabolic and pharmacological effects of the drug in humans and to determine the side effects associated with increasing doses mainly by determining the safety profile. During phase1sufficient information about the dose of the drug ranging studies so that doses for clinical use can be adjusted approximately 70% of drugs tested in this stage move to the next phase.

Phase 2: Phase 2 includes the early controlled clinical studies conducted to obtain some preliminary data on the effectiveness (efficacy) of the potential drug for a particular indication or indication in patients with the disease or condition testing in this phase help to determine the common short-term side effects and risks associated with the drug under testing these studies are typically well-controlled closely monitored and performed on larger groups of patients usually involving 20-300 the length of study vary from several months to 2 years and approximately 33 % of drugs tested as this phase move to the next phase.

Phase3: Phases3 are expanded, controlled and uncontrolled trials the purpose of study at this phase is to gather additional information about the effectiveness and monitoring of adverse reactions phase 3 includes several hundred to several thousand people usually 300 to 3,000 who have the disease or condition to obtain approval from the US FDA it is typically expected that there must be at least two successful phase-3 clinical trials. The length of study varies from 1-4 years. [21,22]

Step 4: FDA Review

Once the new drug is formulated for its best efficacy and safety, and the results from clinical trials are available, it’s advanced for FDA review at this time FDA Review and approves, or does not approve. [23, 24]



Step 5: FDA Post-Market safety monitoring.

This step is also known as Post Marketing Surveillance (PMS) and it is carried out once the candidate drug is approved as a drug and marketed as a medicinal product. This phase aims to find out the drug safety profile in a large patient pool across the world and to establish its Safety profile it is estimated that the success rate of drugs making to market from the laboratory is very less the post-launch safety monitoring helps to detect rare or long term adverse effects of the drugs over a large patient population and time scale than was possible during a clinical trial usually, several thousand volunteers who have the disease or condition are involved in this phase of the trials.[25,26]

Regulatory Affairs in R &D

The Regulatory Affairs personnel work hand in hand with advertising and R & D to develop innovative products that take advantage of new technological and regulatory developments to speed up time to market with new products expected to add great revenues to the company's bottom lines using adaptive clinical trial strategies acquiring quick approval from regulatory authorities and avoiding pitfalls in the method can accelerate the development of new products and help to reduce high-priced errors and time lags.[27,28]

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CONCLUSION

Regulatory Affairs (RA) is a profession that acts as interference between the pharmaceutical enterprise and drug regulatory authorities across the world. It is mainly concerned with the registration of drug products in the respective countries before their marketing. The Regulatory Affairs branch is continually evolving and growing and is the one that is least impacted at some stage in the assembly and addition. Regulatory Affairs departments are growing inside companies. The proper implementation of regulatory guidelines and legal guidelines will improve the economic boom of the company and also improves the security of the people. Regulatory Affairs departments are getting larger inside the companies. Due to the altering resource necessary to fulfill the regulatory requirements, some agencies also choose to outsource or out assignment regulatory affairs to external service providers.

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REGULATORY REQUIREMENTS FOR REGISTRATION OF BIOLOGICS IN US

Akshata J. Patil*, Atish B. Velhal
Department of Pharmacy, Yashoda Technical Campus, Satara, 415003.

ABSTRACT

Biological products are used to treat a wide range of diseases, and the number of biological applications submitted for product approval is on the rise. A biosimilars product's development is more difficult and costly than a small molecule generic product. Biosimilars aren't truly generic medications, but they have a lot in common with the reference biological substance. The Biologics Price Competition and Innovation Act of 2009 established a biosimilar pathway in the United States to enhance access to expensive biological therapies. The research included a "Regulatory Prospect for the Registration of Biological Products in the United States" as well as a summary of the biosimilar product development, manufacturing, and approval process. The regulatory framework, [BLA] Biological License Application, is also discussed in this article.

KEYWORDS: Regulatory, Biologics, BLA, Registration, USA.

1. INTRODUCTION

Biologics are items that are made, extracted, or partly synthesized from a biological source and utilized to prevent, cure, or treat diseases and medical problems. The FDA is in charge of regulating them. • These are large, complex molecules generated by biotechnology in a living system like a bacterium, plant cell, or animal cell, and can be made of carbohydrates, proteins, nucleic acids, or complex combinations of these, or they can be living beings. COPS DSU 3 Department of Pharmaceutics

1.1 The Biologics Control Act of 1902 is a milestone in the history of biologics

Hygienic Lab at PHS.

The National Institutes of Health (NIH) has been renamed (1930)

Control of Biologics at the National Institutes of Health (1937)

1937: The National Institutes of Health (NIH) is established, and the division of biologics is given responsibility for biologics control.

The laboratory of biologics control is renamed in 1944.

The Public Health Service (PHS) Act was enacted in 1944.

The National Institutes of Health's National Microbial Institute is established in 1948, and the laboratory of biologics control is merged into it.

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Later, the institute of Allergy and Infectious Diseases was established.

In 1955, a polio vaccine was inadvertently inactivated

Biological Standards Division, National Institutes of Health

1.2 OBJECTIVE:

The goal of the dissertation work is to have a basic understanding of the "Regulatory requirements for biologics registration in the United States."

Presentation of application forms, their prerequisites, and instructions for filling out and submitting new biologics applications in the U.S

The CTD requirements for registration of biologics

2. REGISTRATION OF BIOLOGICS IN THE USA

Biologics Registration in the United States

In the United States, "biological products" are regulated differently from "drugs" and have various premarket procedures and intellectual property rights. A biological product, on the other hand, must be approved by a biologics license application (BLA) demonstrating that it is "safe, pure, and effective."

A non-biological drug's sponsor must file a new drug application (NDA) demonstrating the drug's potency. The medication is both safe and effective.

The new biological products will be protected for 12 years, whereas the new medications will be protected for only six months.

Protection for up to 5 years. Different strategies for settling conflicts exist in both biological and drug legislation.

Patent difficulties relating to biosimilars and follow-on products. Before a biologic can be used, it must first be approved by the FDA.

The biologic must go through thorough testing before being licensed and marketed.

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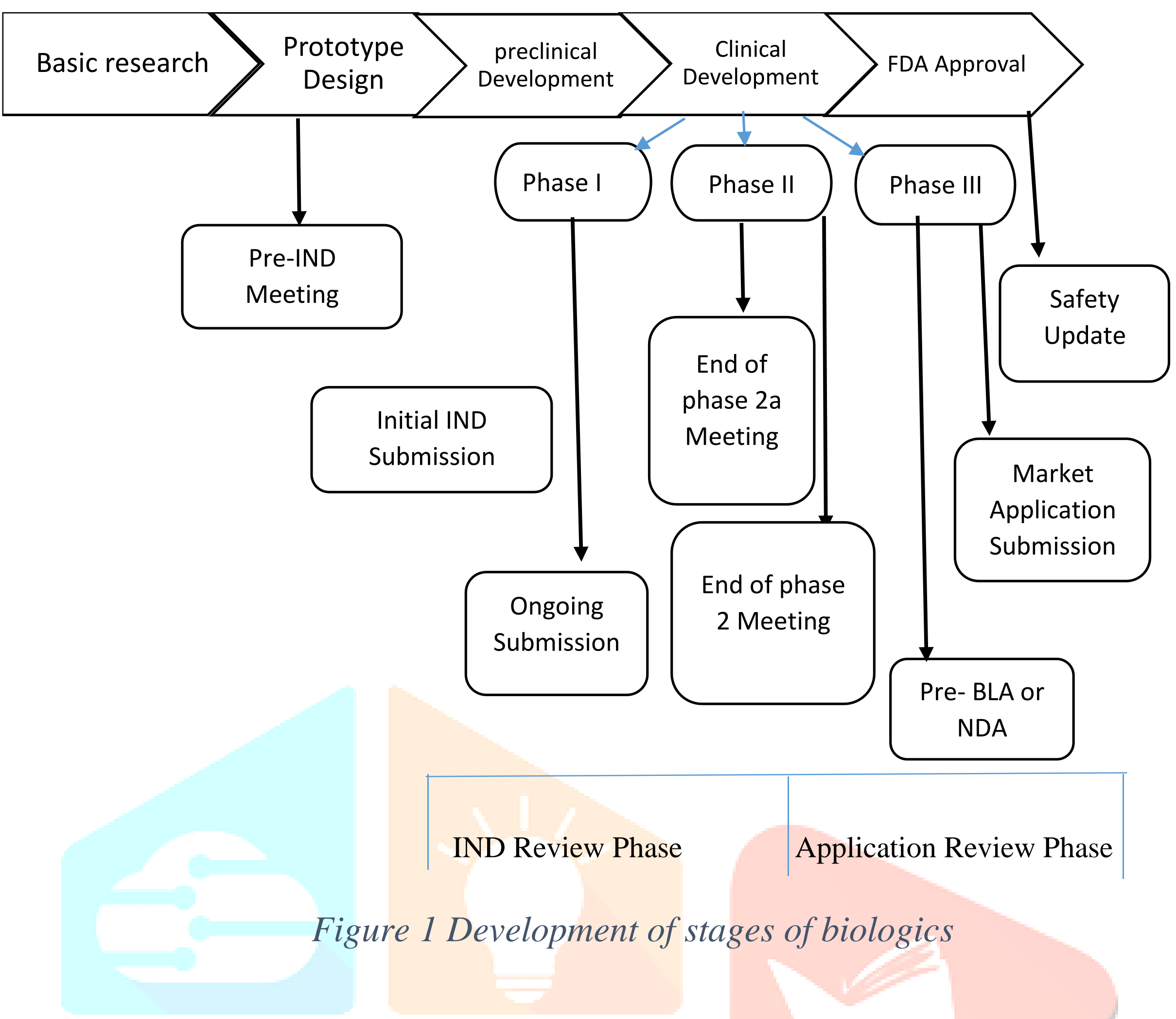

Figure 1 Development of stages of biologics

2.1 BIOLOGICS DEVELOPMENT

BIOLOGICS DEVELOPMENT Living cells or creatures, such as yeasts, viruses, bacteria, or other animal cells, are used to create biologics. A corporation must first demonstrate that it has a viable product to develop before it may create a biological product. This involves a demonstration of the product's ability to be manufactured continuously.

; Figure 1 shows the evolution of biologicals. Preclinical Trial No. 3.1 In vitro and animal experiments are conducted by GLP guidelines. The results of these studies are preclinical data, which are all included in an IND. The FDA reviews the application within 30 days. 3.2

Clinical Research Phase I: A total of 20 to 80 healthy participants were tested for safety and human pharmacology. Phase II: A total of 100 to 200 patients will be tested for basic efficacy and dosing range. Phase III: A multicentre, large-scale investigation involving patients with the target disease

Fundamental research	Discovery	Preclinical Studies	Clinical trials phase I to phase III	FDA Approval

Figure 2: Development of Biologicals

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3. APPROVAL PROCESS

(a) General: To receive a biologics license under section 351 of the Public Health Service Act for any reason, according to 21 CFR 601.2.

The manufacturer of a biological product must submit an application to the Director of the Centre for Biologics.

using forms provided by the Centre for Drug Evaluation and Research or the Director, Centre for Drug Evaluation and Research

for such reasons, and shall submit data resulting from nonclinical laboratory and clinical

l investigations for such purposes

demonstrate that the manufactured product complies with all safety, purity, and potency requirements of each nonclinical laboratory study

The applicant, or his or her attorney, agent, or another representative

The application must be signed by an authorized official.

An application for any of the biological goods listed below that are subject to licensure must include the following information:

(1) Therapeutic DNA Plasmid product

(2) Therapeutic synthetic peptides with 40 amino acids or less.

(3) Monoclonal antibody products for in vivo application; and

(4) Therapeutic recombinant DNA-derived products.

(b) [Reserved]

c) (1) To gain marketing authorization for a therapeutic biological product that is subject to licensure.

A monoclonal antibody, DNA plasmid product, a therapeutic synthetic peptide product of 40 or fewer amino acids

An applicant must submit a product that is either an antibody product for in vivo use or a therapeutic recombinant DNA-derived product

By paragraph, submit a biologics license application

If the requirements of this paragraph

2. conflict with other requirements in this section, the requirements of this paragraph

3. take precedence.

(d) The approval or issue of a biologics license application constitutes a biologics license.

The determination that the establishment(s) and product meet all legal requirements to ensure the continuous safety, purity

(e) As of December 20, 1999, any establishment and product license for a biological product issued under section 351 of the Public Health Service Act (42 U.S.C. 201 et seq.) that has not been revoked or suspended constitutes an approved biologics license application in effect under the same terms and conditions.

terms set out in such product license, as well as those elements of the establishment license relating to such product license

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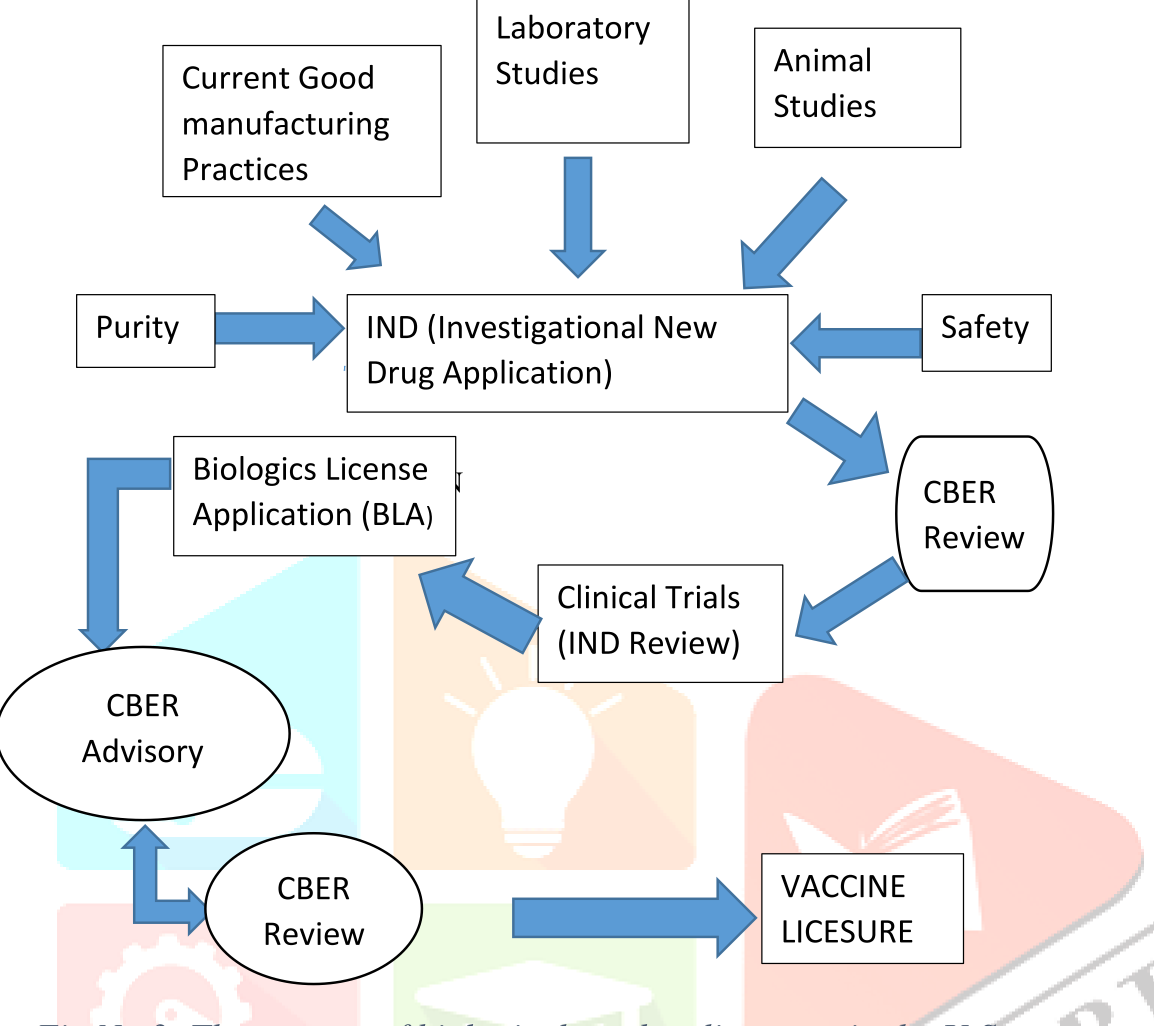

Fig No 3: The process of biological product licensure in the U.S

CONCLUSION:

Biologics are the highly complicated nature medication that is used to treat a variety of diseases and problems. Biotechnology is now being used to create a variety of pharmaceuticals such as antibodies and anticancer medications

Regulatory requirements for registration of new biologics in CTD format according to the FDA include module I which contains administration information, and module II which contains scientific information. module III, in general, contains the quality management system

Moule IV and V contain preclinical and clinical information and information on biologics patent exclusivity the approval process and adjustments made after approval

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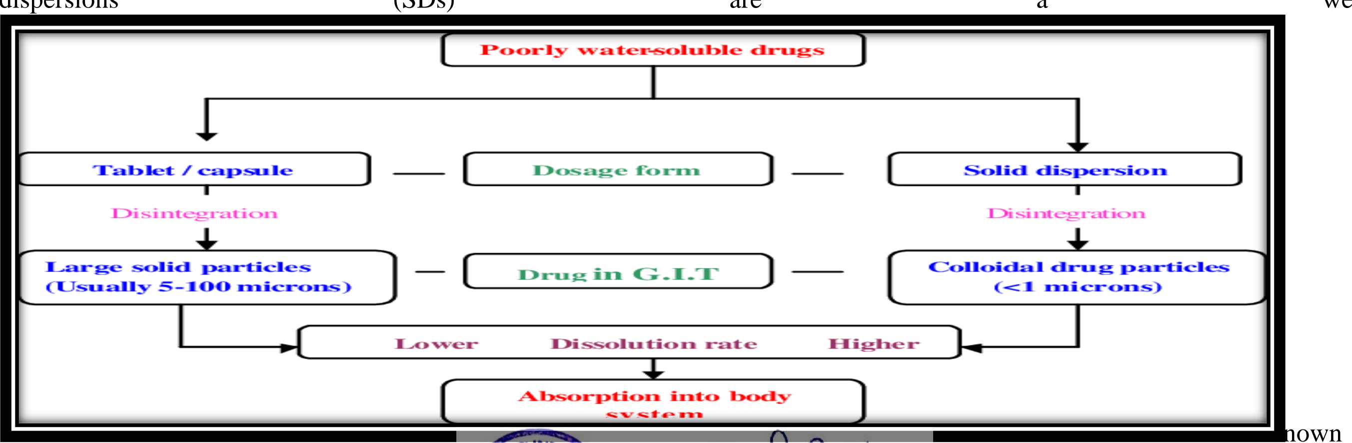
An Outline On Improving Solubility And Dissolution Rate In Solid Dispersion Technique.

[1]Swati.P.Nikam*, [2]Atish.B.Velhal, [3]Prakash.D.Jadhav

YSPM's YTC, Faculty of Pharmacy, Wadhe, Satara, Maharashtra(India). 415004.

Abstract:
To improve dissolution of poorly water-soluble drugs and thus enhancing their bioavailability, the dispersion of one or more active pharmaceutical ingredient in a carrier at solid state is used. This process is known as solid dispersion. It has engrossed significant interest as an efficient means of improving the dissolution rate. Solid dispersions are being employed frequently to improve bioavailability of poorly soluble molecules by enhancing the rate and extent of dissolution in drug product development process. This review discussed the methods for solubility enhancement of poorly soluble drugs and the mechanism by which solubility and dissolution rate enhancement occurs in solid dispersion. The present article also discuss about the manufacturing methods for solubility enhancement, its mechanism and outcome of various low soluble drugs, applications, limitations of the solid dispersions.
***Keywords*: Solid Dispersion, solubility enhancement mechanism, bioavailability.**

INTRODUCTION:
The number of pharmaceutical ingredients (APIs) having low aqueous solubility is currently one of the key issues restricting their biological application. According to estimation, upto 70% of APIs and novel therapeutic entities have poor water solubility, resulting in sluggish absorption and insufficient and unpredictable drug bioavailability [1]. When an active substance is given orally, it must first dissolve in stomach before it can pass through the GI tract's membranes and enter systemic circulation. As a result, a drug with low aqueous solubility will have limited absorption, while a drug with low membrane permeability will have permeation rate limited absorption [2]. As a result, enhancing the bioavailability of active agents by: (i) improving the solubility and dissolution profile of poorly water-soluble medications, and (ii) improving the permeability of drugs. The oral route is the most popular route of administration of the drug due to various reasons like its convenience, good patient acceptance and low medicine production costs [3]. Particle size reduction, salt formation, crystallization, and the use of surfactants and co-solvents are all methods for improving the dissolving capabilities of weakly aqueous-soluble medicines. However, these methods has its own set of constraints, such as the difficulty in forming salts for neutral and weakly acidic/basic medications, and the addition of surfactants/co-solvents leads in liquid formulations with known commercial viability and patient tolerance issues [4]. Furthermore, despite their higher permeability, the majority of potential NCEs are absorbed mostly in the intestine, with absorption dropping substantially after the ileum, showing that absorption is limited [5, 6].
As a result, these medications will have a limited bioavailability if they are not entirely released in this gastrointestinal area. Therefore, improving the water solubility of pharmaceuticals is primary contemporary difficulties facing the pharmaceutical industry [7, 8, 9]. It is feasible to increase bioavailability and prevent side effects by altering the drug release profile of these drugs [10, 11, 12]. Solid dispersions have proven to be most effective ways to enhance the release of low soluble medicines. These are molecular combinations of low aqueous soluble medicines in hydrophilic carriers that have a drug release profile dictated by the polymer characteristics. [13]. Solid dispersion technologies are utilised to improve the solubility properties and, as a result, the bioavailability of low water-soluble compounds. Water insoluble drugs have poor solubility in aqueous gastrointestinal fluids, resulting in insufficient bioavailability. Increased solubility and dissolving rate of the medicine in the gastro-intestinal fluids can improve bioavailability, especially for drugs categorized as Class II by the Biopharmaceutics Classification System. Solid dispersions (SDs) are a well-k



nown approach for improving aqueous solubility and, as a result, oral bioavailability and drug dissolution rate.

Figure 1: Benefits of a solid dispersion formulation over a conventional tablet or capsule formulation [14].

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Applications:

1) The carrier in a solid dispersion plays an important role in enhancing particle wettability. Improved wettability leads to higher solubility, which improves bioavailability [15].
2) Drugs are shown as supersaturated solutions in solid dispersion, which are considered metastable polymorphic forms. Thus, presenting the drug in amorphous form and increases the solubility of the particles [16].
3) Solid dispersions are used for the improvement of the bioavailability of poorly water soluble drugs by enhance the dissolution of the drug [17].
4) Solid dispersions can be formulated as extended release dosage forms.
5) Solid dispersions are better than other particle size reduction methods for improving solubility because other size reduction techniques reduce the size to a limit of about 2-5 microns, which does not result in enough enhancement in drug solubility or drug release in the small intestine, and thus does not improve bioavailability [19].
6) The problems of solid powder such as less size of particles shows poor mechanical properties (include high adhesion and poor flow properties) can be overcome by the use of solid dispersion [19].

Limitations:

1) The polymers employed in solid dispersion can absorb moisture, resulting in phase separation, crystal formation, and the conversion of amorphous to crystalline state [17, 20].
2) Amorphous state of drug undergo crystallization and stability problems.
Most polymers used in solid dispersions absorb moisture, which can cause phase separation, crystal development, or conversion from amorphous to crystalline state, during storage. As a result, the solubility and rate of dissolution may be reduced [21].
3) In the presence of moisture and high temperatures, solid dispersions may degrade.
4) Difficulty in understanding the physical structure of solid dispersions
5) Difficult to determine the shelf life of Solid dispersion.

Approaches for Solubility Enhancement of Poorly Soluble Drug: The techniques that have commonly been used to overcome drawbacks associated with poorly watersoluble drugs, in general includes [17, 18].

Physical Modifications	*Chemical Modifications*	*Others*
Particle size reduction	Salt Formation	Supercritical fluid method
Modification of the crystal habit.	Co-crystallization	Spray freezing into liquid and lyophillization
Complexation	Co-solvency	Hot melt extrusion
Solubilization by surfactants	Hydrotropic	Electrostatic spinning method

THE MECHANISM BY WHICH SOLUBILITY AND DISSOLUTION RATE ENHANCEMENT OCCURS IN SOLID DISPERSION:

Increased Solubility or Dissolution rate of Drug:

Using a variety of carriers may boost the drug's solubility. As a result, the carrier controls the release of the medication, which is independent of the drug's qualities. Additionally, certain systems exhibit release behaviour that is influenced by drug qualities rather than polymer features. The ability of the matrix carrier to enhance drug's local solubility and wettability is also linked to improved solubility dissolution profile of poorly soluble medicines. In his studies, Goldberg et al. investigated the by melting, fully combining, and hardening the mixture of chloramphenicol and urea for additional solubility and dissolution rate investigations, were able to determine the effect of the hydrophilic carrier urea on the solubility of chloramphenicol [21]. As the urea concentration increased from 0% (w/v) to just over 60% (w/v), the solubility of chloramphenicol in the presence of urea increased by more than seven times [21].

Sr.No	*Drug*	*Method of preparation*	*Conclusion*	*Reference.*
1.	(Diazepam and temazepam)	Solvent evaporation and fusion method	Diazepam and temazepam's solubility increases by around 3.5 and 2.5 times, respectively.	[22].
2.	Chloramphenicol	Melting method.	In the presence of urea, chloramphenicol's solubility increased by more than seven times.	[22].
3.	Glibencamide	Anti-solvent addition method	For the manufacture of drug ASD, the co-spray drying approach was used, which greatly improved solubility and resulted in the formation of Glibenclamide-rich amorphous droplets.	[23].

Reduced Particle size [24]:

Size reduction has been considered to be result of eutectic or solid solution formation. Additionally, it has been proposed that presenting the particles in the dissolution media as physically distinct entities may lessen aggregation. Many of the carriers employed in solid dispersion may have wetting capability, which can prevent agglomeration and enhance surface area by improving wetting. When a weakly soluble medication and a highly soluble carrier present in a eutectic mixture are exposed to water or digestive fluid, the highly soluble carrier dissolves, leaving the drug in a fine crystalline form that is easily dissolved. As can be determined from the Noyes-Whitney equation, greater surface area of insoluble chemical results in increased dissolution rate and thus increased oral absorption. Several solid dispersions were documented employing urea as a high water soluble carrier for poorly water soluble medicines such as sulfathiazole, paracetamol, and chloramphenicol. Compared to conventional formulations of the same medications, these solid dispersions exhibited faster release and improved bioavailability. The small particle sizes of the drug played important role in enhancing bioavailability [9,10]. Similarly, because the drug particle size is decreased to an absolute minimum as it is molecularly disseminated in the carrier in a solid solution, it dissolves faster than a eutectic mixture.

Formation of amorphous structure replacing crystalline structure:

Sr.No	*Drug and SD Method*	*Mechanism*	*Conclusion*	*Reference*
1.	Ball Milling (Curcumin)	Particle Size Reduction	The amorphous nature and self-formed micelles of Curcumin SD resulted in a significant improvement in pharmacokinetic behaviour, as illustrated by a 19-fold increase in oral bioavailability..	[25]
2.	Nobiletin	Hot melt extrusion	Amorphous solid dispersion had a greater drug concentration and a 7.5-fold faster dissolving rate. In accelerated stability circumstances, Nobiletin permeability was primarily increased and shown to be stable for up to 6 months.	[26]
3.	Licofelone	Cogrinding mixtures	Enhanced dissolution rate and decreased particle agglomeration	[27]

In the amorphous state, poorly water-soluble crystalline medicines have a higher solubility. The amorphous solids free energy is greater, has specific entropy, and specific volume when compared to corresponding crystalline materials from a thermodynamic standpoint. Amorphous pharmaceuticals have a higher energy state, have the lowest stability, and can be considered as cooled

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liquids. Because the energy necessary to transfer a molecule from a crystal is larger than that required to transfer a molecule from a non-crystalline (amorphous) solid, non-crystalline (amorphous) solids have greater aqueous solubility than crystalline solids. This necessary energy is viewed as an obstacle to medication breakdown. The amorphous state of novobiocin, has ten times the solubility than that of the crystalline form. Chemicals are dissolved or molecularly dispersed in a polymeric carrier in solid molecular dispersions because they lack long-range crystalline structure. The drug is in an amorphous state, which has a higher kinetic solubility and dissolving rate than the crystalline drug (by several orders of magnitude). [7,14,15]. By solvent technique, solid molecular dispersions of diclofenac sodium, naproxen, and piroxicam were generated utilising Poly (2- hydroxyethylmethacrylate) hydrogel as carrier, resulting in the conversion of crystalline drug into amorphous form with enhanced water solubility(16).

Sr. No	***Drug***	***Method of preparation***	***Conclusion***	***Reference***
1.	(Atorvastatin calcium)	solvent evaporation method	The pharmacokinetic study indicated that the Cmax and AUC 0-8h of solid dispersion were improved nearly 2.87-fold and 1.71-fold.Solubility and dissolution rates were enhanced significantly compared with bulk drug	[28]
2.	Vemurafenib	(Micro-precipitated bulk powder technology)	Better dissolution results and a fivefold increase over HPMCAS-L ASD's crystalline form were revealed.	[26]

Complex formation:

In this solid dispersion, in solid state, a drug and an inert soluble carrier form a complex. The solubility and stability constant of the molecule or complex, as well as the drug's absorption rate, determine the drug's availability. It is proposed that the development of a water-soluble compound with a high dissolution constant can increase the dissolution rate and oral absorption. Carbamazepine/PEG 4000 and PEG 6000 solid dispersions, were made using the fusion method, which entails heating a physical mixture of carbamazepine and either PEG 4000 or PEG 6000 to a liquid state. According to dissolving tests, complex formation between carbamazepine and PEG 6000 may be to account for the improvement in solid dispersion dissolution. (27). One of the most frequently used complex carriers are within the class of Cyclodextrins. Cyclodextrins (CD) are cyclic oligomers typically composed of 6–8 glucose units. CDs are a type of solubilizing agent that, by inclusion, produce non-covalent, dynamic complexes with lipophilic molecules.

Sr.No	***Drug and SD Method***	***Mechanism***	***Conclusion***	***Reference No.***
1.	phenacetin (solvent evaporation)	Mechanical Particle size reduction.	The water-soluble hydroxypropylcellulose swells in water and is trapped in the water-insoluble ethylcellulose so that the release of the drug is slowed.This study shows that it is feasible to control PHE release from MC-CP solid dispersions by controlling the complex formation between MC and CP	[26]
2.	Carvedilol	Complexati on and kneading technique	The complexation constant of the medication and the carriers confirmed the formation of stable complexes. The carvedilol had been transformed to an amorphous state, according to solid state data.	[29]

Swelling and capillary action of carrier [24]

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Superdisintegrants like crospovidone, crosslinked polyvinylpyrrolidone etc can considerably used to improve dissolution rate of poorly water soluble drugs. swells 7- to 12-fold in less than 30 sec. Croscarmellose swells 4- to 8-fold in less than 10 seconds in two dimensions, retaining fibre length equal.This indicates that rate, force, and extent of swelling have an important role in disintegrants that work by swelling.

By improving porosity solid dispersion [24]:

The porosity of particles in solid dispersions has been discovered to be greater. Porosity increases with carrier qualities; eg Solid dispersions with linear polymers produce larger, more porous particles than those with reticular polymers, resulting in a faster dissolving rate.

Interactions of the drug with Carrier functional groups:

In addition to improving the drug's local solubility and wettability, carrier matrix also helps to improve the medication's aqueous

Sr.No	**Drug and SD Method**	**Mechanism**	**Conclusion**	**Reference No.**
1.	AZD0837	Hot melt extrusion	The molecule remained amorphous throughout the dissolving process and was kept in a super saturated and stable condition, according to the findings.	[30]
2.	Indomethacin (IND),	Hot melt extrusion	BCS Class II medication that benefits from the addition of a porous carrier to a ternary mixture and exhibits better dissolving capabilities than the drug-polymer binary mixture alone.	[26]

solubility and dissolving rate through specific interactions with the drug. [19,20]

a) The intermolecular hydrogen bonding:
b) By elevating the Tg(transition temperature) of the solid dispersion mixtures:
c) Inhibited drug precipitation from supersaturated solution:
d) By formation of Metastable drug polymorphous with improved solubility and dissolution rate:

Sr.No	**Drug and SD Method**	**Mechanism**	**Conclusion**	**Reference**
1.	**Rivaroxaban**	Melt quenching approach	Physical stability of prepared ASDs was aided by intermolecular interactions with moisture.	[31]
2.	**Griseofulvin**	Freeze drying	Because of its high degree of supersaturation and high crystallisation propensity, there was a significant improvement in dissolving and oral absorption.	[32]
3.	**Itraconazole**	Solvent evaporation	In comparison to PVPVA, HPMCAS demonstrated excellent storage stability at RH levels greater than 60%, which can be attributed to its greater glass transition temperature and lower hydrophobicity.	[26]

4.	**Etoposide**	Solvent evaporation	According to experimental studies, the solubility of etoposide above the Critical micellar concentration (CMC) grew linearly, and the ASD permitted super saturation. By boosting P-gp saturation, a high level of super saturation via ASD increased the drug's in-vivo permeability.	[26]
5.	**Curcumin**	Solvent evaporation	Because of the hydrogen bond interaction between the curcumin and the polymer, HPMC E5 has a substantial impact on crystallisation inhibition and enhanced the permeability of the amorphous drug..	[32]

Conclusion:

Solid dispersion systems have been realized as extremely useful tool in improving the dissolution properties of poorly water-soluble drugs. In recent years, a great deal of knowledge has been accumulated about solid dispersion technology, but their commercial application is limited. Various methods have been tried recently to overcome the limitation and make the preparation practically feasible. The problems involved in incorporating into formulation of dosage forms have been gradually resolved with the advent of alternative strategies.

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EVALUATION OF ANTICATALEPTIC ACTIVITY OF BACLOFEN ON HALOPERIDOL & PILOCARPINE INDUCED CATALEPSY

Shriram D. Virkar*, Atish B. Velhal, Vitthal J. Chaware and Vivekumar K. Redasani

Department of Pharmacology, YSPM's Yashoda Technical Campus Wadhe, Satara, India.

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***Corresponding Author**
Shriram D. Virkar
Department of Pharmacology, YSPM's Yashoda Technical Campus Wadhe, Satara, India.
shriraamvirkar@gmail.com

ABSTRACT

Aim: Evaluation of Anticataleptic activity of baclofen on Haloperidol & Pilocarpine induced catalepsy. **Materials and methods:** A cataleptic behaviour was measured with a high bar test method. Catalepsy score was measured each hour for 4 h after haloperidol and pilocarpine administration, by gently placing both the forepaws of the rat over a metal bar (diameter 2–5 mm) situated 6 cm above the tabletop. The intensity of catalepsy was assessed by counting the time in seconds until the rat brought both forepaws down to the tabletop, with a maximum cut-off time of 180s. **Result:** Haloperidol 1mg/kg p.o and Pilocarpine 100mg/kg p.o caused a time dependent increase in catalepsy, with maximum score occurring at 4 hours after administration. Animal treated by Baclofen (10mg/kg) p.o could significantly reduce catalepctic score as compared to toxicant control animals. **Conclusion:** It is concluded that Baclofen at the dose of 10mg/kg p.o can possess anticatalepctic effect.

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1. INTRODUCTION

Catalepsy is a condition characterized by inactivity, decreased responsiveness to stimuli and a tendency to maintain an immobile posture. It may be associated with the nervous system drug toxicity, psychotic disorders and other conditions. Catalepsy is the neurodegenerative disease of unknown etiology and characterized by motor symptoms of tremor, rigidity, bradykinesia, and postural instability. Catalepsy is characterized by an abnormal basal ganglia activity. Nonmotor comorbidities, such as cognitive impairments (the comorbidity of anxiety and depression like Parkinson's disease) are likely the result of an intricate interplay of multi-system degenerations and neurotransmitter deficiencies extending beyond the loss of dopaminergic nigral neurons (costall,B and Naylor 1974).

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Haloperidol is an antipsychotic drug, which is used in the treatment of schizophrenia and other psychotic disorders. Antipsychotics are often associated with distressing extrapyramidal side effects. Haloperidol-induced catalepsy occurs due to the blockade of dopamine (D2) receptors and reduced dopaminergic transmission. The phenomenon of cataleptic immobility induced in rodents by typical neuroleptics (e.g. haloperidol) is a robust behavioural model to study nigrostriatal function and its modulation by cholinergic, serotonergic, nitrergic and other neurotransmitter systems.

Baclofen (P-p-chlorophenyl GABA) is used clinically in the treatment of spasm of voluntary muscles. Its mode of action has been attributed to the facilitation of GABA-mediated transmission in the central nervous system. Baclofen would therefore be expected to inhibit the activity of nigro-striatal dopaminergic neurones and in the process potentiate the cataleptogenic effect of haloperidol. Gianutsos & Moore (1977) reported that baclofen produced a dose-dependent increase in the concentration of brain dopamine in mice without affecting noradrenaline levels. They suggested that this effect was achieved by a reduction in impulse flow in dopaminergic neurones in a similar way to that produced by y-butyrolactone (GBL), (Roth, Walters, Murrin &Morgenroth, 1975). This study examines the effect of Baclofen on the cataleptogenic effect of haloperidol.

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2. MATERIALS AND METHODS

2.1 Animals and housing condition: The experiment was conducted with Wistar male rats of 150-200 g and 2–3 months old. Female rats are excluded from the present study since estrogen has been reported to possess neuroprotective property and this might mask development of Catalepsy. These animals were procured from registered breeder and were acquainted in the quarantine area for one week. After acquaintance, animals were transferred to the standard of22±2°C temperature,50±15%relative humidity, 12hr.dark/12hr. light)cycle and the animals had free access to pellet diet & water was provided *ad libitum*. The study protocol was presented to the IAEC for approval.

2.2 Study design

Haloperidol induced catalepsy

The 36 male albino wistar rats were divided into 6 groups: group I received vehicle, group II received Haloperidol (1mg/kg), group III received Haloperidol (1mg/kg) and Levodopa(100mg/kg), group IV received Haloperidol(1mg/kg) and Baclofen(5mg/kg), group

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V received Haloperidol(1mg/kg) and Baclofen(10mg/kg), group VI received Haloperidol(1mg/kg) and Baclofen(15mg/kg).

Pilocarpine induced catalepsy

The 36 male albino wistar rats were divided into 6 groups: group I received vehicle, group II received Pilocarpine(100mg/kg), group III received Pilocarpine(100mg/kg) and Levodopa(100mg/kg), group IV received Pilocarpine (1mg/kg) and Baclofen(5mg/kg), group V received Pilocarpine (100mg/kg) and Baclofen(10mg/kg), group VI received Pilocarpine (100mg/kg) and Baclofen(15mg/kg).

2.3 Experimental procedure: A cataleptic behaviour was measured with a high bar test method. Catalepsy score was measured each hour for 4 h after haloperidol and Pilocarpine administration, by gently placing both the forepaws of the rat over a metal bar (diameter 2–5 mm) situated 6 cm above the tabletop. The intensity of catalepsy was assessed by counting the time in seconds until the rat brought both forepaws down to the tabletop, with a maximum cut-off time of 180 s. Finally, scores at different time points (0, 30, 60, 120, 180 and 240 min after haloperidol and Pilocarpine administration) was added and expressed as a cumulative catalepsy score for comparison purpose.

2.4 Scoring of catalepsy

Cataleptic animal maintaining this position for a period of time dependent upon the degree of catalepsy. If the animal maintained the imposed posture for at least 20s it was said to be cataleptic and given one point. Scoring is modified from that used by Costall and Naylor (1974). Animals maintaining the cataleptic posture from 0 s to 10 s scored 0; 10 s to 30 s = 1; 30 s to l min=2; l min to 2 min=3; 2 min to 3 min=4; 3 min to ∞ =5. Animals were tested for catalepsy 0.5, 1.0, 2.0,3.0 and 4.0h after haloperidol and Pilocarpine administration (Costall& Naylor, 1974).

2.5 Statistical Analysis: The data obtained by the various parameters was statistically evaluated by one way analysis (ANOVA) followed by Dunnett's multiple comparison test by graph pad prism software (Graph pad software Inc.…5.0.0). The mean values±SEM were calculated for each parameter. Level of significance was kept at $p<0.05$.

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3. RESULTS

3.1 Effect of Baclofen on cataleptic score using High bar Test

Table 1: Haloperidol induced catalepsy.

Groups	Dose	30 min	60 min	120 min	180 min	240 min
Normal control(vehicle)	---	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Negative control (Haloperidol)	1mg/kg p.o	0.0±0.0	0.0±0.0	3.16±0.28	4.66±0.43	4.83±0.47
Positive control (Haloperidol+ Levodopa)	1mg/kg p.o +100mg/kg p.o	0.0±0.0	0.0±0.0	0.83±0.08	0.66±0.04	0.00±0.00
Low dose (Haloperidol+ Baclofen)	1mg/kg p.o +5mg/kg p.o	0.0±0.0	0.0±0.0	1.83±0.15	1.66±0.12	1.66±0.15
Intermediate dose (Haloperidol+ Baclofen)	1mg/kg p.o +10mg/kg p.o	0.0±0.0	0.0±0.0	0.66±0.05	0.66±0.03	0.66±0.04
High dose (Haloperidol+ Baclofen)	1mg/kg p.o +15mg/kg p.o	0.0±0.0	0.0±0.0	4.66±0.41	4.63±0.41	4.63±0.40

All value expressed as mean ± SEM; n=6 rats in each group, by one way ANOVA followed by Dunnett's multiple comparison test (compared with toxicant control) $p<0.05$.

1. At 30 min, 60 min, 120 min, 180 min, 240 min animals treated with Haloperidol 1mg/kg showed significant increase in number of catalepsy as compared to control group.
2. At 30 min, 60 min, 120 min, 180 min, 240 min animals treated with Levodopa 100 mg/kg showed significant decrease in number of catalepsy score as compared to negative group (Haloperidol 1mg/kg).
3. At 30 min, 60 min, 120 min, 180 min, 240 min animals treated with Baclofen (5mg/kg)showed significant decrease in number of catalepsy score as compared to negative group (Haloperidol 1mg/kg).
4. At 30 min, 60 min, 120 min, 180 min, 240 min animals treated with Baclofen (10mg/kg)showed significant decrease in number of catalepsy score as compared to negative group (Haloperidol 1mg/kg).
5. At 30 min, 60 min, 120 min, 180 min, 240 min animals treated with Baclofen (15mg/kg) did not reduce catalepsy score as compared to negative group (Haloperidol 1mg/kg).

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Table 2: Pilocarpine induced catalepsy.

Groups	Dose	30 min	60 min	120 min	180 min	240 min
Normal control(vehicle)	---	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Negative control (Pilocarpine)	100mg/kg p.o	0.0±0.0	1.55±0.13	2.33±0.20	3.16±0.28	3.66±0.32
Positive control (Pilocarpine + Levodopa)	100mg/kg p.o+100mg/kg p.o	0.0±0.0	0.64±0.06	0.57±0.05	0.55±0.06	0.55±0.04
Low dose (Pilocarpine + Baclofen)	100mg/kg p.o+5mg/kg p.o	0.0±0.0	1.12±0.10	1.06±0.08	1.03±0.12	1.03±0.11
Intermediate dose(Pilocarpine + Baclofen)	100mg/kg p.o+10mg/kg p.o	0.0±0.0	0.64±0.04	0.55±0.05	0.55±0.06	0.53±0.04
High dose (Pilocarpine + Baclofen)	100mg/kg p.o+15mg/kg p.o	0.0±0.0	3.55±0.30	3.83±0.38	4.66±0.40	4.83±0.45

All value expressed as mean ± SEM; n=6 rats in each group, by one way ANOVA followed by Dunnett's multiple comparison test (compared with toxicant control) $p<0.05$.

1. At 30 min, 60 min, 120 min, 180 min, 240 min animals treated with Pilocarpine 100mg/kg showed significant increase in number of catalepsy as compared to control group.
2. At 30 min, 60 min, 120 min, 180 min, 240 min animals treated with Levodopa 100 mg/kg showed significant decrease in number of catalepsy score as compared to negative group (Pilocarpine 100mg/kg).
3. At 30 min, 60 min, 120 min, 180 min, 240 min animals treated with Baclofen (5mg/kg)showed significant decrease in number of catalepsy score as compared to negative group (Pilocarpine 100mg/kg).
4. At 30 min, 60 min, 120 min, 180 min, 240 min animals treated with Baclofen (10mg/kg)showed significant decrease in number of catalepsy score as compared to negative group (Pilocarpine 100mg/kg).
5. At 30 min, 60 min, 120 min, 180 min, 240 min animals treated with Baclofen (15mg/kg) did not reduce catalepsy score as compared to negative group (Pilocarpine 100mg/kg).

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3.2 Effect of baclofen on locomotor activity using actophotometer

Table 3: Haloperidol induced catalepsy.

Groups	**Treatment(dose)**	**Locomotor activity counts(10 min)**
Group I(Normal control)	Vehicle	482.0±13.91
Group II(negative control)	Haloperidol(1mg/kg)	267.0±11.87
Group III(positive control)	Haloperidol(1mg/kg)+Levodopa(100mg/kg)	422.5±11.78
Group IV(Test I)	Haloperidol(1mg/kg)+Baclofen(5mg/kg)	311.5±12.11
Group V (Test II)	Haloperidol(1mg/kg)+Baclofen(10mg/kg)	350.8±12.40
Group VI (Test III)	Haloperidol(1mg/kg)+Baclofen(15mg/kg)	232.0±13.82

All value expressed as mean ± SEM; n=6 rats in each group, by one way ANOVA followed by Dunnett's multiple comparison test (compared with toxicant control) $p<0.05$.

1. Negative group (Haloperidol 1mg/kg) showed significant decrease in locomotor activity as compared to normal control group.
2. Animals treated with Levodopa 100mg/kg(positive control) showed significant increase in locomotor activity as compared to negative group.
3. Animals treated with Baclofen (5mg/kg) showed significant increase locomotor activity as compared to negative group (Haloperidol 1mg/kg).
4. Animals treated with Baclofen (10mg/kg) showed significant increase locomotor activity as compared to negative group (Haloperidol 1mg/kg).
5. Animals treated with Baclofen (15mg/kg) did not show significant locomotor activity as compared to negative group (Haloperidol 1mg/kg).

Table 4: Pilocarpine induced catalepsy.

Groups	**Treatment(dose)**	**Locomotor activity counts(10min)**
Group I (Normal control)	Vehicle	468.8±10.07
Group II (Negative control)	Pilocarpine(100mg/kg)	143.7±12.48
Group III (Positive control)	Pilocarpine(100mg/kg)+Levodopa(100mg/kg)	442±13.84
Group IV (Test I)	Pilocarpine(100mg/kg)+Baclofen(5mg/kg)	335.6±12.35
Group V (Test II)	Pilocarpine(100mg/kg)+Baclofen(10mg/kg)	360.7±12.95
Group VI (Test III)	Pilocarpine(100mg/kg)+Baclofen(15mg/kg)	242.3±14.72

All value expressed as mean ± SEM; n=6 rats in each group, by one way ANOVA followed by Dunnett's multiple comparison test (compared with toxicant control) $p<0.05$.

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1. Negative group (Pilocarpine 100mg/kg showed significant decrease in locomotor activity as compared to normal control group.
2. Animals treated with positive control Levodopa 100mg/kg(positive control) showed significant increase in locomotor activity as compared to negative group.
3. Animals treated with Baclofen (5mg/kg) showed significant increase locomotor activity as compared to negative group (Pilocarpine 100mg/kg).
4. Animals treated with Baclofen (10mg/kg) showed significant increase locomotor activity as compared to negative group (Pilocarpine 100mg/kg).
5. Animals treated with Baclofen (15mg/kg) did not show significant locomotor activity as compared to negative group (Pilocarpine 100mg/kg).

4. DISCUSSION

Catalepsy can be produced by blocking dopaminergic striatal pathways or by stimulating the cholinergic pathways (Liisa Ahtee). At striatal dopaminergic receptors, catalepsy has been linked to a functional absence of dopamine(Van Rossum 1966).By inhibiting post-synaptic dopamine receptors, neuroleptics like haloperidol produce this effect. Additionally, it's probable that haloperidol functions as a "feedback" blocker of presynaptic or autoreceptors that control dopamine production (Fuxe, Hokfelt, Ljung-dahl, Agnati, 1975). Dopamine turnover is enhanced overall, and dopamine receptor stimulation is inhibited. Pilocarpine increased the striatal homovanillic acid (HVA) content up to three times the original concentration. According to reports, baclofen inhibits the rise in dopamine turnover caused by neuroleptics, most likely by slowing down the firing rate of dopaminergic neurons and making them less sensitive to neuronal "feedback" mechanisms. It has been proposed that baclofen increases the concentration of brain dopamine in a dose-dependent manner, whereby the reduction in impulse flow in dopaminergic neurones results in an increased activation of tyrosine hydroxylase. (Gianutsos and moore 1977). Since a GABA-ergic system has been hypothesised that inhibits the nigro-striatal dopaminergic system, it would be expected that increasing endogenous brain GABA levels or potentiating GABA-ergic transmission would increase the cataleptogenic action of neuroleptics.

In this study, two animal models were used actophotometer and high bar test. All the models are widely accepted behaviour models for assessing pharmacological anticataleptic activity. The results described in this paper agree with the above findings in that there is a potentiation of catalepsy up to 60 minutes. However, we found that after 60 min the cataleptogenic effect

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of haloperidol and pilocarpine was attenuated and remained significantly so when compared to control animals for up to 4 hours. It would appear that the facilitation of GABA-ergic inhibition on the nigro-striatal dopaminergic neurons produced by baclofen diminishes after 60 min. We propose that the reversal of catalepsy by baclofen is a result of the reininstatement of impulse flow in the nigro-striatal dopaminergic neuronal system (J.A Davies and J.Williams).

5. CONCLUSION

It is concluded that Baclofen at the dose of 5mg/kg p.o can possess anticatalepctic effect. Baclofen at the dose of 15 mg/kg did not reduce cataleptic score as compare to toxicant control animals. The effect was more prominent when animals treated with Baclofen (10mg/kg).

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Evaluation of protective role of a Ferulic acid on Letrozole induced polycystic ovarian syndrome in female rats

Karishma M. Yadav[1]*, Priyanka K. Ghadage[1], Rupali V. Bhoite[1], Prajakta B. Phadtare[1], Omkar A. Devade[2]

[1]Department of Pharmacology, YSPM's Yashoda Technical Campus, Wadhe, Satara, India.
[2]Department of Pharmacology, AISSMS College of Pharmacy, Pune - 411001, India.

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ABSTRACT: Background: *Ferulic* (hydroxycinnamic) *acid* is an antioxidant of phenolic phytochemical group used for the skin care product. Polycystic Ovarian Syndrome (PCOS) is a state of hormonal disorder causing an enlarged ovary with small cysts at the outer edges. Aim: The study was designed to investigate the protective effect of ferulic acid (3-methoxy-4-hydroxycinnamic acid) in letrozole induced polycystic ovarian syndrome in rats (PCOS). Methods: All the experimental animals except control group were orally administered with Letrozole (1mg/kg) dissolved in 0.5 % w/v Carboxymethyl cellulose (CMC) solution per oral route for 21 days to induce PCOS. Followed by a dose of ferulic acid (10, 20, and 40 mg/kg p.o.) for 15 days using water as vehicle. Results: The PCOS was confirmed in the letrozole induced rats with increased concentration of androgen, abnormal lipid levels, glucose, glycosylated haemoglobin and also depletion of antioxidants. The administrated of letrozole cause to abnormalities in serum hormone profile, lipid profile, blood glucose levels and increases body weight and ovary weight. Ferulic acid successfully exerted its protective effect by restoring all the parameters to normalize and improving or disappearance of ovarian cysts. Histopathological observations showed a remarkable recovery of the ovarian tissue and the presence of normalized structure of antral follicle. Conclusion: Ferulic acid showed protective effects in letrozole induced PCOS in rats. Biological effects of ferulic acid make it a promising drug for treating clinical and pathological abnormalities against PCOS conditions.

Corresponding author
Ms. Karishma M. Yadav,
YSPM's Yashoda Technical Campus,
Wadhe, Satara, India.
Tel: +91-8767445487
E. Mail ID: y.karishma53@gmail.com

Keywords: PCOS, Fertility; Ovulation, Letrozole, Ferulic acid, Cysts.

INTRODUCTION:

Polycystic ovary syndrome (PCOS) is a common and complex female endocrine disorder in women of reproductive age [1,2] with an estimated prevalence of 6 to 10 % [3]. Clinical manifestation of PCOS amenorrhea, abdominal obesity, hirsutism, and androgen excess (Hyperandrogenism), infertility, and expanded ovaries with multiple cysts. Women with PCOS are at increased risk for diabetes, dyslipidemia, atherosclerosis,

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bleeding, hypertension, cardiovascular disease as well as endometrial carcinoma [4]. It is also related with psychological impairments like depression and related mood disorders.

Lipid imbalance, insulin resistance, oxidative stress, and genetics are some of the contributing factors of PCOS [5]. Currently, many therapies are available to induce ovulation and manage PCOS, but it is associated with mild to severe side effects, like; arthritis, hot flushes, muscle or joint pain and psychological side effects like, mood swings, depression, irritability, and bloating. Therefore now-a-days focus is being laid on natural source herbal medicinal plants that have been utilized for the treatment of the various disorders related to the reproductive system due to the lesser or no side effects [3].

Ferulic acid(2*E*)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid) is water soluble, phenolic compound found in active chemical constituent in Chinese medicine herbs such as female ginseng ,and many staple foods, like; fruits, cereals, vegetables and coffee [6,7]. Ferulic acid has been reported to possess a wide variety of biological effects like Antioxidant, anti-inflammatory, hypoglycaemic, and Hyperlipidemic activities [8]. In this study we evaluated that Ferulic acid (3-methoxy-4-hydroxycinnamic acid) may be beneficial in management of PCOS induced by Letrozole due to the reported activity.

MATERIALS:

Drugs and reagents:

Letrozole and Clomiphene citrate were purchased from retail Shop Satara, India. Ferulic acid was obtained from Dolphin Pharmacy Instruments, Pvt., Ltd. Mumbai.

METHODS:

In this study the experimental models used is Letrozole induced PCOS models. The model was widely used accepted for assessing PCOS activity. All animals were selected and divided into six groups and housed eight female rats per cage. All animals in five groups except control group were orally administered with Letrozole for 21 days.

Two animals from each group were scarified by using CO_2 chamber. Ovaries was removed and observed for presence of cysts. On 22nd day, Test group I, II, and III was administered with Ferulic acid for 15 days, whereas standard group was dosed with Clomiphene citrate for 15 days per oral route [9-11].

Animals:

This prospective comparative study was conducted at Department of Pharmacology, YSPM's Yashoda Technical Campus, Wadhe, Satara, and Maharashtra, India. Healthy, Virgin, cyclic and adult female wistar rats (150 to 200 g) were used in the present study. These animals were procured from registered breeder and acquainted in the quarantine area for one week.

Housing of animals:

The animals were housed in polypropylene cages with paddy husk as bedding. The animals were maintained under standard laboratory conditions of 22 ± 2°C temperatures, 50 ± 15 % of relative humidity, 12 h dark/ 12 h light cycle with free access to pellet diet and water provided *ad libitum*. The study protocol was approved form institutional animal ethic committee. The experiments were performed as per as guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Governments of India. The Institutional Animal Ethics Committee approved the study protocol YSPM/YTC/PHARMA-IAEC/48/2020.

PCOS induction:

All the experimental animals except control group were orally administered with letrozole (1 mg/kg) dissolved in 0.5 % w/v CMC solution per oral route for 21 days to induce PCOS. Vaginal smear checked or examined daily and the animals in regular estrous phase were selected for study. Vaginal smears were collected and evaluated microscopically using Crystal violet stain to confirm the induction of PCOS. Two animals from each group were scarified by using CO_2 chamber. Ovaries were removed and observed for presence of cysts [11,12]. In female rats, the estrous cycle characterized by proestrus, estrus, metestrus (or diestrus I) and diestrus (or diestrusII) in normal animals. During estrus cyclic differences in vaginal cytology occurs in response to the morphological changes and continuous changes in cell types (leukocytes, nucleated epithelial and cornified epithelial) occurs in PCOS induced animals [8,9].

Treatment groups:

Animals were randomly assigned into six group (Table 1) and adequate supply food and drinking water.

Study design:

The study consisted of 48 female Albino Wistar rats equally divided into 6 groups as group 1 (control

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group), group 2 (PCOS induced group), group 3 (Standard group), group 4, 5, and 6 as treatment groups. Following Letrozole administration, standard group was administered with Clomiphene citrate at a dose of 1mg/kg in 0.5 % CMC per oral and treatment group 4, 5, and 6 were administered Ferulic acid with the dose of 10, 20, and 40 mg/kg of body weight respectively in water per oral for 15 days. After 21 days, PCOS control group and after 36 days, animals from other groups were fasted overnight and blood was collected by retro orbital puncture then serum was separated and was used for estimation of hormones, lipid parameters and glucose. Body weight was measured at the end of study (On day 36th) animals were then sacrificed and ovaries were excised, cleaned of fat and weighed [11].

Table 1. Treatment Groups.

Group	Treatment
Group 1: Control	Healthy rats were administered vehicle (10 ml/kg)
Group 2: Negative control	Animals were administered with Letrozole (1 mg/kg)
Group 3: Positive control	Animals were administered with Letrozole (1 mg/kg) + Clomiphene citrate (1 mg/kg)
Group 4: Test group with low dose	Animals were administered with Letrozole (1 mg/kg) + Ferulic acid (10 mg/kg)
Group 5: Test group with intermediate dose	Animals were administered with Letrozole (1 mg/kg) + Ferulic acid (20 mg/kg)
Group 6: Test group with high dose	Animals were administered with Letrozole (1 mg/kg) + Ferulic acid (40 mg/kg)

Biochemical estimation:

***Measurement of fasting blood glucose*:**

Blood glucose level was measured by using Accu-cheak active glucometer (Roche Diabetes care GmbH Sandhofer Strasse11668305 Mannheim, Germany).

***Hormonal assay*:**

Blood samples were collected by retro-orbital puncture; serum was used for hormonal estimation (FSH, LH and Testosterone). Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), Testosterone was measured via Enzyme Linked Immunosorbent Assay (ELISA) with the help of commercial kits (ELISA kit).

***Lipid profile*:**

The lipid profile (LDL, HDL, Total cholesterol, Triglycerides) was estimated at the end of the study. Lipid profile (LDL, HDL, Total cholesterol, Triglycerides) were quantified by using enzymatic kits procured from Aspen Laboratories pvt, Ltd

***Histopathology*:**

The excised ovaries were fixed in 10 % v/v formalin solution. According to histological procedure, they were subjected to tissue processing by washing with water which was followed by dehydration through ascending grades of alcohol then cleared through xylene. Then paraffin embedding method was used. The blocks were sectioned by using microtome and were placed on slides. These sections were stained with hematoxylene-eosin (HE), dehydrate, cleared and mounted on DPX mount under glass cover slips. The light microscope was used for observation which was connected to a camera to capture image.

Statistical analysis:

The statistical analysis was done by using Graph pad software version 5.0 and results were compared by one-way ANOVA followed by Tukey's Multiple Comparison Test. The results were analysed by Two-way analysis of variance followed by Bonferroni posttests. A p value <0.05 was considered as statistically significant.

RESULTS:

Examination of oestrus cycle:

Fig 1. showed oestrus cycle phase of animals. Displaying oestrous cycle stage only animals with a regular cycle were used for research, Fig 2 demonstrated not observed cornified squamous epithelial cells (Crystal violet staining) in PCOS induced groups.

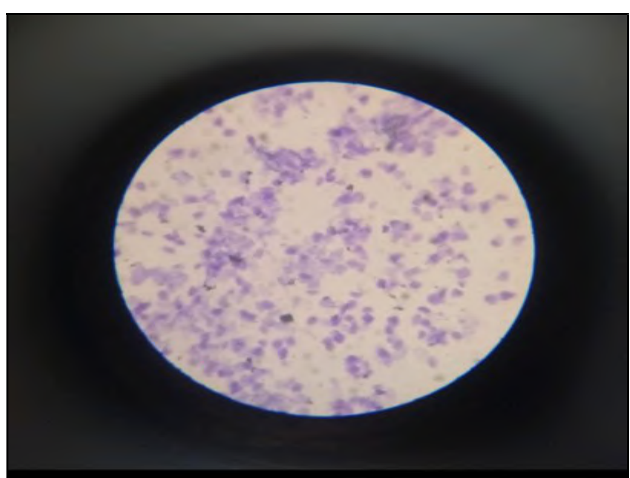

Fig 1. Smear with cornified squamous epithelial cells (Normal animals).

Showed oestrus cycle phase of animals. Displaying oestrous cycle stage only animals with a regular cycle were used for research.

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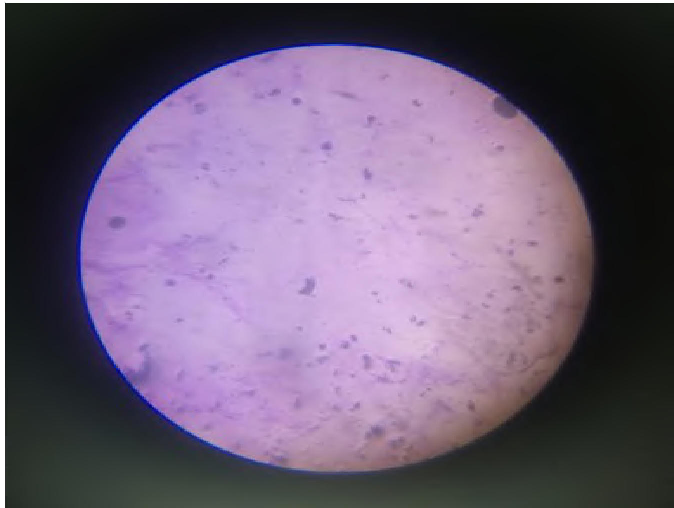

Fig 2. Examination of oestrus cycle (PCOS induced animals).
Not observed cornified squamous epithelial cells (Crystal violet staining) in PCOS induced groups.

Morphology of ovary:

Fig 3 shows Normal ovary structure, where as Fig 4 shows Fluid filled cysts in PCOS induced group.

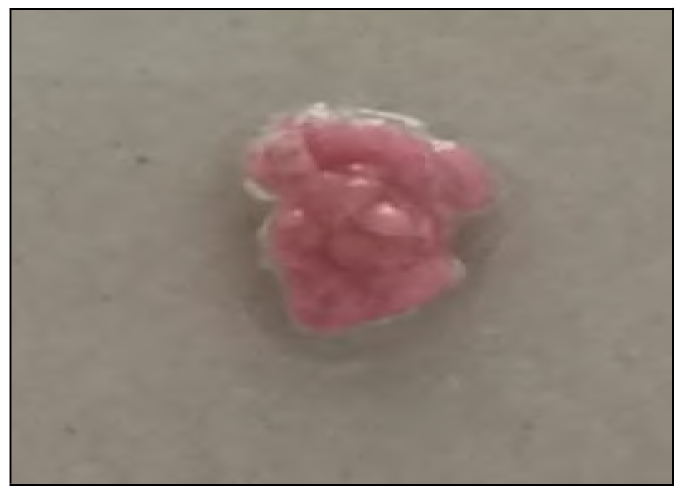

Fig 3. Morphology of ovary (Normal ovary).

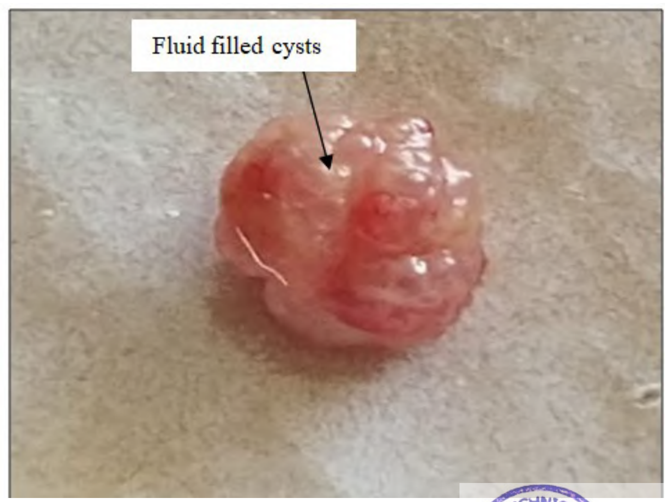

Fig 4. Morphology of ovary (Fluid filled cysts in PCOS induced group).

Body weight:

The effect of Ferulic acid on body weight was represented in Fig 5. Letrozole treatment to a significantly increase in body weight ($p<0.001$) as compared to control group. Oral treatment with Ferulic acid at dose of 10, 20, 40 mg/kg, for 2 weeks ($P<0.001$, $P<0.001$ and $P<0.001$; respectively) significantly reduced the body weight in experimental animals while treatment with Clomiphene citrate (1 mg/kg) significantly decreased ($P<0.001$) body weight when compared to Negative control rats.

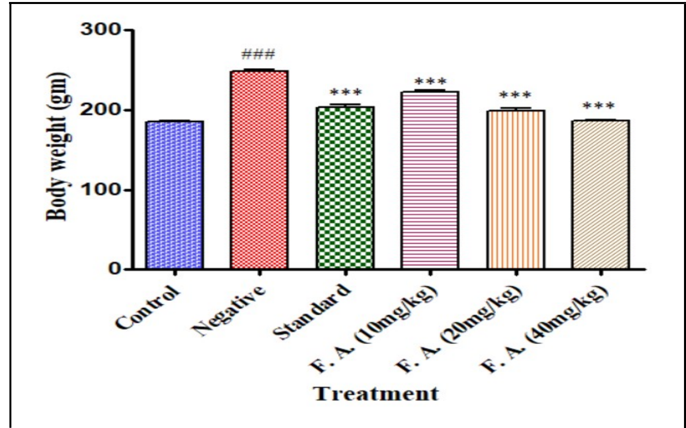

Fig 5. The effect of Ferulic acid on body weight.
All values represent mean ±SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. ###$p<0.001$; when compared with normal control. ***$p<0.001$; when compared with negative control.

Organ weight:

Letrozole treatment to a significantly increase in ovarian weight ($p<0.001$) as compared to control group. Oral treatment with Ferulic acid at dose of 10, 20, 40 mg/kg, for 2 weeks ($P<0.01$, $P<0.001$ and $P<0.001$; respectively) significantly reduced the ovary weight in experimental animals while treatment with Clomiphene citrate (1 mg/kg) significantly decrease ($P<0.001$) ovary weight when compared to Negative control rats as given in Fig 6.

Serum hormonal profile:

The serum levels of Testosterone and luteinizing hormone (LH) were increased in PCOS induced group ($p<0.001$, $p<0.001$; respectively) while follicle stimulating hormone significantly decreased ($p<0.001$) in comparison to the control group. A significant fall ($p<0.001$) in testosterone levels was observed in standard, low dose, intermediate dose and high dose groups. Treatment with at dose of Ferulic acid 10, 20, 40 mg/kg and standard ($P<0.01$, $p<0.01$, $p<0.001$, and

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Table 2. The effect of Ferulic acid on serum hormonal level.

Groups	Testosterone (ng/ml)	LH (ng/ml)	FSH (ng/ml)
Control	0.092 ± 0.003	12.17 ± 0.70	25.67 ± 2.72
Negative	0.140 ± 0.003###	19.33 ± 1.25###	10.50 ± 0.99###
Standard	0.112 ± 0.001***	11.17 ± 0.60***	21.67 ± 0.80***
F. A. (10 mg/kg)	0.119 ± 0.002***	15.0 ± 0.68**	15.33 ± 1.11
F. A. (20 mg/kg)	0.092 ± 0.002***	14.50 ± 0.76**	17.50 ± 0.99*
F. A. (40 mg/kg)	0.083 ± 0.002***	11.17± 0.60***	20.17 ± 0.60***

Note: All values represent mean ±SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. ###p<0.001; when compared with normal control. *p<0.05, **p<0.01, ***p<0.001; when compared with negative control. LH and FSH are luteinizing and follicular stimulating hormone.

Table 3. The effect of Ferulic acid on lipid profile.

Groups	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Triglyceride (mg/dL)
Control	61 ± 1.65	26 ± 1.18	22.17 ± 1.30	82.50 ± 1.97
Negative	102 ± 2.58###	14.67 ± 0.66###	51.17 ± 2.10###	132.80 ± 2.82###
Standard	76.67 ± 1.74***	22.67 ± 0.88***	38.67 ± 0.88***	90.83 ± 2.57***
F. A. (10mg/kg)	90.67 ± 1.97**	19.17 ± 1.07*	41.50 ± 0.76**	109.70 ± 2.48***
F. A. (20mg/kg)	71.17 ± 1.35***	21.50 ± 0.76***	37.17 ± 1.32***	90.67 ± 1.97***
F. A. (40mg/kg)	62.50 ± 1.89***	27.67 ± 0.88***	26.67 ± 2.33***	75.67 ± 2.96***

Note: All values represent mean ±SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. ###p<0.001; when compared with normal control. *p<0.05, **p<0.01, ***p<0.001; when compared with negative control.

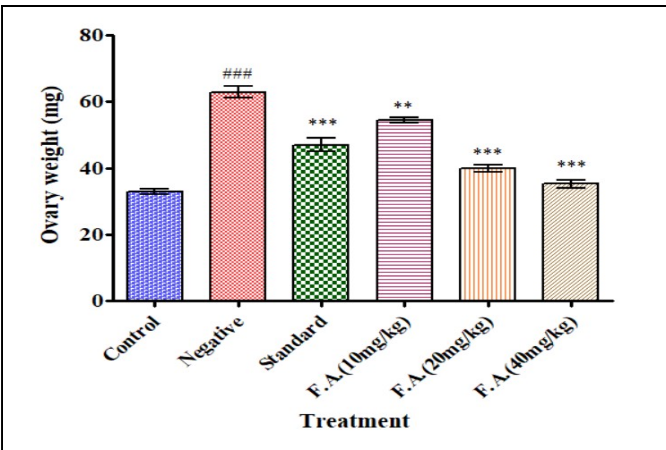

Fig 6. The effect of Ferulic acid on ovarian weight.
All values represent mean ±SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. ###p<0.001; when compared with normal control. **p<0.01, ***p<0.001; when compared with negative control.

p<0.001; respectively) produced a significant decreased in Luteinizing hormone levels when compared with Negative group. Animals treated with at dose of Ferulic acid 20, 40 mg/kg and standard produced a significant increase (p<0.05, p<0.05, and P<0.001; respectively) in FSH levels when compared with Negative group (Table 2).

Ferulic acid reduces blood glucose level:

The effect of Ferulic acid on blood glucose levels was represented in Fig 7. Letrozole treatment to a significantly increase in blood glucose levels (p<0.001) as compared to control group. Oral treatment with at dose of Ferulic acid 10, 20, 40 mg/kg, for 2 weeks (P<0.001, P<0.001 and P<0.001; respectively) significantly decreased the blood glucose levels in experimental animals while treatment with Clomiphene citrate (1mg/kg) significantly decrease (P<0.001) blood glucose levels when compared to Negative control rats.

Lipid profile:

The effect of Ferulic acid on serum lipid profile was represented in Table 3. Letrozole treatment showed

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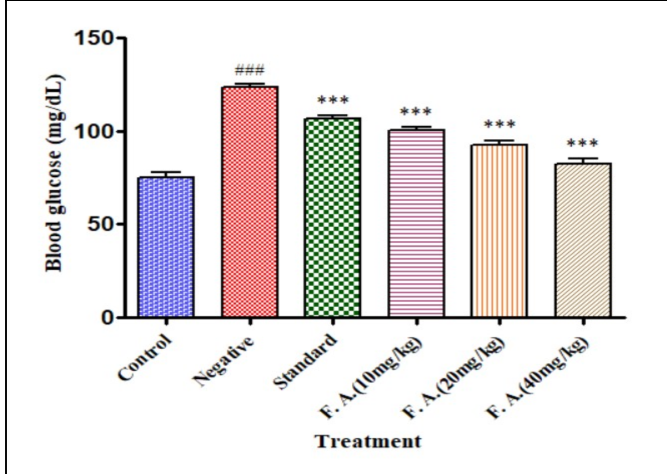

Fig 7. Ferulic acid reduces blood glucose level.
Note: All values represent mean ±SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. ###p<0.001; when compared with normal control. ***p<0.001; when compared with negative control.

Lipid profile:
The effect of Ferulic acid on serum lipid profile was represented in Table 3. Letrozole treatment showed significant changes in serum lipid as compared to control. Cholesterol, LDL and triglyceride were greatly increased as p<0.001, p<0.001 and p<0.001 respectively while HDL levels were decreased (p<0.001) in PCOS induced group (Negative group). Clomiphene treatment significantly decreased Cholesterol (p<0.001), LDL (p<0.001) and triglyceride (p<0.001) levels when compared to PCOS induced group. While HDL levels significantly increased (p<0.001) when compared to PCOS induced group. Low dose of Ferulic acid (10 mg/kg) decreased the levels of Cholesterol (p<0.01), LDL (p<0.01) and triglyceride (p<0.001). It also increased HDL level significantly (p<0.05) in comparison to negative group. Intermediate dose of Ferulic acid (20 mg/kg) decreased the levels of Cholesterol (p<0.001), LDL (p<0.001) and triglyceride (p<0.001). It also increased HDL level significantly (p<0.001) in comparison to negative group. High dose of Ferulic acid (40 mg/kg) decreased the levels of Cholesterol (p<0.001), LDL (p<0.001) and triglyceride (p<0.001). It also increased HDL level significantly (p<0.001) in comparison to negative group.

Histomorphological changes
Histopathological examination of stained sections of ovary showed ovarian changes and ovarian follicular cysts (Fig 8). Yellow coloured arrow showing numbers of ovarian follicular cysts. Negative group showing multiple numbers of ovarian follicular cysts compared to normal control group. Oral administration of Clomiphene citrate (1 mg/kg), low dose of Ferulic acid (10 mg/kg), Intermediate dose of Ferulic acid (20 mg/kg), and high dose of Ferulic acid (40 mg/kg) significantly improved or disappearance the number of ovarian follicular cysts compared to negative group.

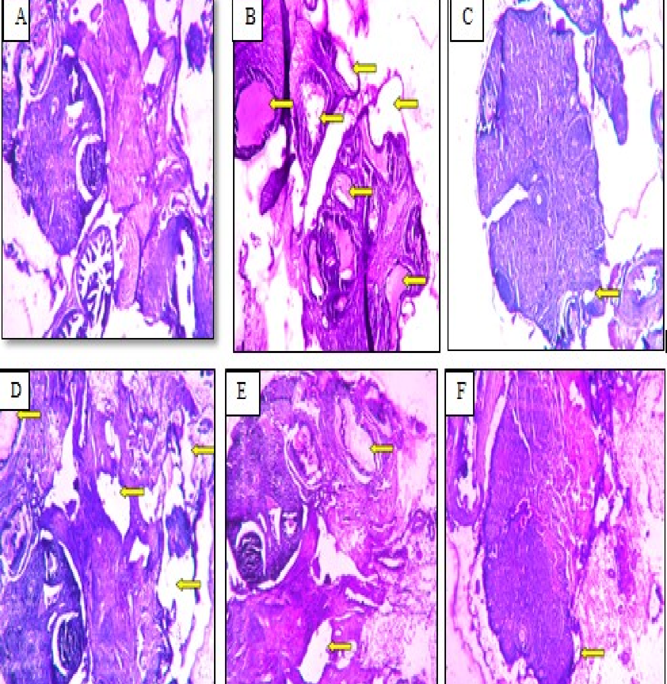

Fig 8. Effect of Ferulic acid in HE-stained ovary tissue (40X).
A. Normal control: showing normal histology of ovary. B. PCOS control: showing large numbers of ovarian follicular cysts. Yellow arrow indicates cysts. C. Letrozole + Clomiphene citrate showing less numbers of cysts. Yellow arrow indicates cysts. D. Letrozole + Ferulic acid (10 mg/kg) showing fewer moderate numbers of cysts. Yellow arrow indicates cysts. E. Letrozole + Ferulic acid (20 mg/kg) showing less numbers of cysts. Yellow arrow indicates cysts. F. Letrozole + Ferulic acid (40 mg/kg) showing less numbers of cysts. Yellow arrow indicates cysts.

DISCUSSION:
Polycystic ovarian syndrome (PCOS) is major female health problem. It is a chronic metabolic disorder characterized by hyperglycaemia, obesity, excess androgen level, hyperlipidaemia, and decrease FSH level. The World Health Organization estimates that it affects 116 million women worldwide as of 2012 [13]. Various experimental models for PCOS have been developed in rats like administration of testosterone propionate (TP), dehydroepiandrosterone (DHEA), and 5a-dihydrotestoterone (DHT) and Estradiol valerate (EV). It is models fully convincing and identify with the

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condition of human PCOS completely [14]. Letrozole is a non-steroidal aromatase inhibitor that reduces conversion of androgens to estrogens in the ovary, resulting increased testosterone and decreased E2 production and stimulate PCOS like condition by causing circulating hyperandrogenism, hormonal imbalance, and intra ovarian androgen excess leading to appearance of polycystic ovary. Letrozole induced PCOS was causehyperglycaemic condition which may contribute to insulin resistance, hyperlipidaemia leading to metabolic syndrome [10-15]. Letrozole induce animal model causes polycystic ovarian syndrome in our research study. It is PCOS rat model characterized by an increase in androgen biosynthesis. P450 aromatase enzyme is responsible converting testosterone and androstenedione to estradiol and estrone. This enzyme inhibits activity led to enhance ovarian androgen production or concentration and resulted in PCOS disorder. Due to inhibit of aromatase enzyme activity increases ovarian androgen secretion and resulted into increase level or concentration of testosterone, LH, and FSH, Letrozole treatment showed some metabolic feature, like increased body fat, triglycerides, cholesterol and body weight [10,14]. Ferulic acid showed marked significantly decreased body weight and ovary weight in PCOS rats that may be responsible for reduced the fatty formation, decreasing follicular cysts (follicular fluid). The body weight was considerably reduced by treatment with Ferulic acid (20 and 40 mg/kg). The weight of ovaries in the negative control group was greater than that of normal control group rats. Ferulic acid (20 and 40 mg/kg) treatment significantly decreased ovaries weights which matched to those in control group animals. Type-2 diabetic mellitus and insulin resistant hyperglycaemia are inter-linked with PCOS. Altered insulin levels which can directly stimulate ovarian androgen production in PCOS Insulin stimulate adrenal steroidogenesis by enhancing sensitivity to adrenocorticotrophic hormone (ACTH) and increase pituitary LH release. Increase androgen level cause ovarian cyst. FA improves altered insulin levels, impaired glucose homeostasis and insulin sensitivity [15]. PCOS induced rats showed marked rise in blood glucose level relative to control group. Oral administration of Ferulic acid significantly reduced the increased blood sugar levels, and indicating the beneficial impact of Ferulic acid on insulin resistance and diabetic condition. Women with PCOS are hyperandrogenemic which is associated with alteration in circulating lipoprotein and lipid level resulting in dyslipidemia. Regulation of carbohydrate metabolism, insulin plays important role in the metabolism of lipids. Insulin is inhibitor of lipolysis, since it inhibits the activity of the hormone-sensitive lipases in adipose tissue and increased FFA concentration into the circulation. Increased FFA concentration also raises β-oxidation of fatty acids, producing more acetyl-CoA and cholesterol. FA decreased the levels of FFA, TG, Cholesterol and phospholipids in plasma [16-19]. Characteristically PCOS patient have increased cholesterol level. The women with PCOS tend to be obese probably due to high cholesterol and lipid content. The same effect was seen in current research work after PCOS induction. In comparison with the normal control group, the negative control group reported significantly enhanced LDL, Cholesterol, triglycerides concentration and lowered HDL concentration. Ferulic acid (10, 20, and 40 mg/kg) decreased significantly LDL, cholesterol, triglycerides levels and enhanced HDL level. Ferulic acid displayed beneficial outcome against hyperlipidaemia. In this research, non-steroidal aromatase inhibitor Letrozole blocks the conversion of testosterone to estradiol. This lead in testosterone and LH level increased while FSH level decreased. This imbalanced hormonal level leads to inconsistent oestrus cycle [20,21]. The similar condition has been noted in our research. Letrozole induced rats showed considerably increased levels of testosterone, LH and decreased FSH levels compared to control. Standard drug Clomiphene citrate (1 mg/kg), and Ferulic acid (20 and 40 mg/kg) treated rats showed significantly decreased testosterone, LH level and FSH level increased. The Histopathological report of Letrozole induced rats indicated the existence of polycysts in the ovary. Negative group showed large numbers of ovarian follicular cysts. After treatment with Ferulic acid (20 and 40 mg/kg), decreased or improved numbers of ovarian follicular cysts. All the biochemical and Histopathological parameters in our results advocate the Ferulic acid is most constructive treatment against PCOS.

CONCLUSION:

Treating the various parameters in PCOS induced rats, the impact of Ferulic acid treatment with intermediate (20 mg/kg) and high (40 mg/kg) dose was observed to be similar with standard treatment (Clomiphene citrate). In Letrozole induced PCOS animals, Ferulic acid restored the lipid profile, hormone and glycemic status

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as well as ovarian morphology. Ferulic acid might be beneficial in managing PCOS condition due to multiple pharmacological actions like hypoglycemic effects, antihyperlipidemic, anti-inflammatory, protective action against obesity, phytoestrogenic and antioxidant activity. Biological effects of Ferulic acid make it a promising drug for treating clinical and pathological abnormalities against PCOS condition.

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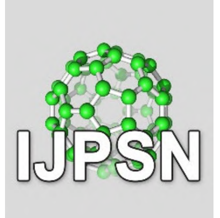

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RESEARCH ARTICLE

Role of Aminated Derivatives of Natural Gum in Release Modulating Matrix System of Losartan Potassium

Shankar B. Kalbhare[1&3*] | Rohit K. Pawar[5] | Dr. Vivekkumar K. Redasani[2] | Amita B. Yadav[1] | Vishal R. Mohite[1] | Vaibhav B. Kadam[4]

[1]Assistant professor (Department of Pharmaceutics), YSPM's, Yashoda Technical Campus, Satara, India 415001;
[2]Director, YSPM's, Yashoda Technical Campus, Satara, India 415001;
[3]Research & Development Officer, Khandelwal Laboratories Pvt. Ltd. Thane India 400604;
[4]Research & Development Executive, Khandelwal Laboratories Pvt. Ltd. Thane India 400604;
[5]Assistant professor (Department of Pharmaceutics), Late Narayandas Bhawandas Chhabada Institute of Pharmacy, Raigaon, Satara, India 415020.

***Correspondence author:** Shankar B. Kalbhare*, Assistant professor (Department of Pharmaceutics), YSPM's, Yashoda Technical Campus, Satara, India 415001
Phone: 7507571444
Email id: kirankal786@gmail.com
https://orcid.org/0000-0002-1176-5207

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ABSTRACT

Objective: The investigation aimed to synthesize amino derivatives of various natural gums like Xanthan gum and Tamarind gum for using them as a release modulating polymer in the formulation of the hydrophilic matrix system of losartan potassium and to find the best amongst them. Developing oral sustained release matrix tablets for a drug with a constant release rate has always been a challenge to the pharmaceutical technologist.

Materials and Methods: Release modulating hydrophilic matrix tablets of losartan potassium were prepared by wet granulation method. A total number of 6 formulations of release modulating hydrophilic matrix tablets of losartan potassium were prepared using different polymeric ratios of Carbopol 934, aminated Tamarind gum and aminated Xanthan gum based on preliminary trial bathes. The formulated tablets were evaluated for both pre-compression and post-compression evaluation studies.

Results: Based on in vitro drug release study the effective formulations AXG 3 are shows a maximum similar release profile to other remains formulations with a theoretical drug release profile of losartan potassium for sustained release. Finally optimized formulation AXG 3 containing carbopol 934 (60 mg), aminated xanthan gum (40 mg), MCC (190 mg) and magnesium stearate (10 mg) showed 100±0.024 % drug release in 12 hr which is acceptable with theoretical drug release of losartan potassium for sustain release dose. Conclusion: Aminated derivatives of xanthan gum and Tamarind gum extend the drug release for 12 hr. Based on in vitro drug release studies of formulations, we concluded that the alteration in the concentration of carbopol 934 with an aminated derivative of xanthan gum in sustain release formulation development was more effective and economical.

Keywords
Release modulating matrix tablets; Amination of natural polymers; Aminated xanthan gum; Aminated Tamarind gum; Losartan potassium; Theoretical drug release profile

ABBREVIATIONS: *LSP: losartan potassium; IRD; Immediate release dose; CDR: Cumulative drug release; TDRP: theoretical drug release profile*

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Introduction

Polymers are stable on chemical modification(Deore & Khadabadi, 2008). Several chemical modification methods of natural gums have been reported, including amination of Tamarind gum and amination of Xanthan gum reaction, both utilizing the reactivity of hydroxyl groups on the natural polymers(Goswami & Naik, 2014). In the present investigational study, a novel two-step derivatisation procedure was taken on to make aminated tamarind and Xanthan gum. Newly derivatized aminated natural gum contains a huge portion of 1^0 and 2^0 amine groups. The modified natural polymer's derivatives have many potential applications(Chivate et al., 2008). Both modified natural gums derivatives are reactive species in preparation for the release modulation matrix system of the BCS II class drug(Dioscorus et al., 2014; Shukla et al., 2018). Aminated derivatisation of natural polymers increases bioadhesive and drug-release properties(Bassi & Kaur, 2015). In recent times chemical modifications of natural polysaccharides were used to improve the functional properties of natural gums(Ahmad et al., 2019). Reports in the literature show that derivatives of polysaccharides like amine can be used to increase swelling & bioadhesion properties and also drug release (Jain et al., 2008; Mankala et al., 2011; Yeole et al., 2006).

Tamarind gum and Xanthan is neutral, nontoxic and very stable to heat, pH, and shear changes and has near Newtonian flow behaviour (Dey et al., 2011). A Chemical or synthetic transformation procedure of amination is applied to ameliorate the performance of these uncontrollable but reasonable polysaccharides. Cationic polysaccharides have capacious usefulness in drug and gene delivery, and new areas like biologics and fluorescent labelling applications. Hence to meliorate the effectiveness of the Tamarind and Xanthan gum, a cationic structural transformation for complete amination was implemented for synthesis and some of its properties are described in this article. Aminated Tamarind and aminted Xanthan have been developed to enhance the biological characteristics of the native Tamarind gum and Xanthan gum including biocompatibility, hydrophilicity, bioadhesive property, and antibacterial activity. The authors have developed release-modulating matrix systems by using aminated Tamarind and aminted Xanthan combined as efficient carriers for the delivery of anti-hypertensive (losartan potassium) drugs.

Losartan potassium (LSP) is a potent and highly specific angiotensin II type I receptor agonist having antihypertensive activity. LSP was having about 33% of oral bioavailability and plasma elimination half-life in the 1.5 to 2.5 hrs in range(Chopra et al., 2007). Upon administration of LSP in the form of controlled release drug delivery combined with two types of release characteristics which firstly burst release and then extended release over 8hrs(Debnath et al., 2011). These characteristics would be more desirable because they would allow the rapid onset of action and subsequently gives a prolonged antihypertensive effect with the help of maintaining plasma drug concentration above therapeutic concentration(Kalbhare et al., 2020; R. Kumar et al., 2009)

Derivatisation of polymers by the introduction of functional groups leads to an increase in the bioadhesive strength of the derivatized polymer as compared to the inhabitant polymer(Dey et al., 2011). For example, thiolated trimethyl chitosan nanoparticles exhibited 2.1 to 4.7 fold greater mucoadhesion than trimethyl chitosan nanoparticles. These have also been utilized as viscosity enhancers, stabilizers, disintegrates, solubilises, emulsifiers suspending agents, gelling agents and bioadhesive and binders in the above-mentioned dosage form. A sustained drug delivery system of BCS II class drugs has significantly improved the therapeutic efficacy of drugs by using aminted derivatized natural gum(Dumortier et al., 2006). The modified amino derivatives show better antimicrobial activity in comparison to chitosan. Aminated Tamarind and Aminted Xanthan gum are many explanations for the effectiveness of medication dosage forms to improve the bioavailability of the designed dosage form and decrease the frequency of administration to prolong the period of successful blood levels. Modified aminated derivatives of Tamarind gum and Xanthan gum also decrease peak variability by concentration and side effects, and probably improve the effective delivery of the Losartan potassium (LSP).

Materials and Methods

Materials

Losartan potassium (LSP) was provided as a gift sample by Viraj Pharmaceutical. (Mumbai, India). Carbopol 934, magnesium stearate and microcrystalline cellulose were supplied by Thermosil Fine Chem. industry. (Charhol. Tamarind gum and xanthan gum were purchased from Phyto Life sciences Pvt Ltd. (Gandhinagar, Gujarat). Starch, isopropyl alcohol and ethylene diamine are supplied by SD. Fine Chemicals limited. (Mumbai, India). Sodium borohydride was purchased from Karan enterprise. (Mumbai, India). All other reagents and solvents used were of analytical grade and used as received.

Methods

Amination of natural polymers

In 3000 ml water add 60 gm of natural gum. To this solution add aminating agent ethylene diamine (25 ml) with continuous stirring at constant temperature (20-60°C) for (6 hr). Then slowly add the reducing agent sodium borohydride (NaBH4) for 2 hr until the formation of a thick gel. Wash this gel several times with ethyl alcohol and collect the precipitate of aminated derivative. The synthesized aminated polymer was studied under further parameters for the determination of flow

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properties, chemical stability and thermal properties(Liu et al., 2008).

Calculation of theoretical drug release profile and fixation of dose

The theoretical sustained release drug profile was calculated based on the pharmacokinetic parameters of the drug. The immediate release dose (IRD) of the drug (drug release at 1st hr) was calculated using the following formula (1).

$$IRD = \frac{C_{ss} \times V_d}{F} \quad \ldots (1)$$

Where Vd-Volume of distribution; F-Bioavability; C_{ss}-Steady state concentration

Study of release behaviour of aminated derivatives

To assure the consistency of the premix blend, the drug, Carbopol 934 as a binder, and the MCC as a diluent were physically sifted through #40 and well blended. The chosen quantity of derivatized polymers (Aminated TG and Aminated XG), previously passed through #40, were then combined with several drug-diluent premixes for 5 minutes. With the aid of isopropyl alcohol, the premix mixture was wetly granulated. The granules were then sized through #18 and dried at 45 °C for 15 min. Starch and magnesium stearate was used to lubricate the dried LSP granules. The tablets were compressed using the cold compression technique on a calibrated hydraulic press (KBR press) with 12.0 mm, round, flat punches at compressional pressure of 5 tones with a dwell duration of 15 seconds. The average compression weight of the compressed tablets was 400 mg.

Formulation development

Different formulations of Losartan potassium (LSP) 100 mg release modulating matrix tablets were prepared using the following excipients: Aminated TG (30–40 mg), Aminated XG (30–40 mg), Carbopol 934 (60-70 mg), starch (10.25 mg), magnesium stearate (10 mg) and MCC (q.s. to 190 mg). The amounts of ATG and AXG used to prepare each of the 3 in different concentrations, formulations are given in Table no 2.

To maintain the consistency of the premix blend, the drug, Carbopol 934 (as a binder), and the MCC (as a diluent) were physically sifted through #40 and properly blended. The chosen ratio and combinations of hydrophilic polymers (Aminated TG and Aminated XG), which had been previously sorted through #40, were then blended with several drug premixes for 5 minutes. With the aid of isopropyl alcohol, the premix mixture was wetly granulated. The granules were then sized through #18 and dried at 45 °C for 15 min. Starch and magnesium stearate was used to lubricate the dried LSP granules. The tablets were compressed using a cold compression method on a dialed hydraulic press (KBR press) with punches that were 12.0 mm in diameter and flat with a compressional pressure of 5 tones and a dwell duration of 15 seconds(Chopra et al., 2007; Larsen et al., 1973; Mandal et al., 2007).

Table 1. Formulation table for trial batches

Ingredient	Batches				
	T1	T2	T3	T4	T5
LSP* (mg)	100	100	100	100	100
Carbopol 934(mg)	100	75	75	50	50
ATG** (mg)	-	25	-	50	-
AXG*** (mg)	-	-	25	-	50
Microcrystalline cellulose (mg)	190	190	190	190	190
Magnesium Stearate (mg)	10	10	10	10	10
Total weight tablet (mg)	**400**	**400**	**400**	**400**	**400**

Losartan potassium; **Aminated Tamarind gum; * Aminated Xanthan gum*

Table 2. Formulation table of developed formulations

Ingredient	Batches					
	ATG1	ATG2	ATG3	AXG1	AXG2	AXG3
LSP	100	100	100	100	100	100
Carbopol 934	70	65	60	70	65	60
ATG	30	35	40	-	-	-
AXG	-	-	-	30	35	40
Magnesium Stearate	10	10	10	10	10	10
Microcrystalline cellulose	190	190	190	190	190	190
The total weight (mg)	400	400	400	400	**400**	**400**

*All values expressed in milligram (mg).

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Both trial formulation and developed formulations were studied under further parameters for determination of flow properties, post compression parameters and in vitro drug release study. In vitro drug release profile was compared with therapeutic drug release profile of LSP for sustain release dose. The formulated batch shows maximum similarity with the therapeutic drug release profile which the batch considered for optimization.

Uniformity of drug content

Five tablets of different formulations were weighed separately and powdered. The powder equivalent to the average weight of tablets was weighed and the drug was extracted in methanol, the drug content was determined by measuring the absorbance at 217.0 nm after suitable dilution using a UV Visible Spectrophotometer (UV-1800)(Basu et al., 2011; Yeole et al., 2006).

In vitro drug release studies

Dissolution studies were performed in triplicate, trying to maintain the sink conditions for all the preparations. A 5 ml equal volume of sample was withdrawn at appropriate intervals, filtered and analyzed spectrophotometrically at 205.3 nm. For the formulations, the total per cent drug release was quantified and the drug release data were curve-fitted using MS-excel to evaluate the possible drug release mechanism from swollen hydrophilic matrices(Y. G. Kumar et al., 2013; Mandal et al., 2007).

Swelling and erosion studies

The tablets were slashed, and gently started cleaning using tissue paper to remove surface water after 2 h in 0.1 N HCl and 6 h in phosphate buffer pH6.8, and a Scanning Electron Microscopy (SEM) analysis of the hydrated swelling tablets was performed(Hu et al., 2007). Water uptake and mass loss were determined gravimetrically according to the following equations,

$$\text{Degree of swelling (water uptake)} = \frac{\text{Wet weight} - \text{Original dry weight}}{\text{Original dry weight}} \quad \text{.....(2)}$$

$$\text{Erosion (\% mass loss)} = \frac{\text{Original weight} - \text{Remaining dry weight}}{\text{Original weight}} \quad \text{.....(3)}$$

Results

Flow properties and physicochemical evaluation of aminated polymers

The bulk density, tapped density and angle of repose of synthesized polymers were increased as compared to natural polymers due to the reason of chemical modification was done in the natural polymer. The compressibility index and Hausner's ratio describe the flow properties of natural polymers and derivatized polymers. Observations as per compressibility index the aminated Tamarind gum shows poor flow properties as compared to natural Tamarind gum. In the case of aminated xanthan gum compressibility index is partially increased and shows good flow properties. The values of Hausner's ratio are < 1.25, which shows good flow. Here all derivatized polymers show good flow properties except aminated Tamarind gum. Evaluation parameters like bulk density, tapped density, angle of repose, compressibility and Hausner's ratio were carried out for the natural polymers and derivatized polymers and were found to be within the limit as given in Table no 3.

Compatibility study of ATG and AXG with Drug

The synthesized modified aminted derivative of Tamarind and Xanthan is characterized using ATR-FTIR, DSC and XRD studies. In this study, synthesis polymers are confirmed by the ATR-FTIR study shown in figure 1. The ATR- FTIR study of aminated Tamarind gum and aminated xanthan gum is confirmed by the appearance of a new peak at 3271 cm^{-1}, 1639.49 cm^{-1} and 2899.01 cm^{-1} respectively corresponding to the NH_2 group. ATG shows an endothermic peak at 82.40°C and 394.50°C with the heat of flow of 1.002 w/g and 9.231 w/g respectively shown in figure 2 (I). The AXG shows an endothermic peak at 71.74°C and 541.66°C with the heat of flow of 1.644 w/g and 9.424 w/g. All amine derivatives of natural polymers do not show an exothermic peak in DSC thermograms due to the loss of water content in the polymer. The diffraction curve of ATG and AXG was typical of amorphous material with no sharp peaks shown in figure 2 (II).

Calculation of theoretical drug release profile and fixation of sustain release dose

Immediate release dose of drug represents predicted fraction of drug release in 1h was calculated using the formula (1)

$$\text{IRD} = \frac{C_{ss} \times V_d}{F}$$

Where C_{ss} represents steady-state concentration which is calculated by the following formula

$$C_{ss} = \frac{F \times D}{CL \times \tau}$$

Where CL= clearance (liter/kg); D = conventional single dose (50 mg); τ = dosing interval (100 mg bid = 2 h)

Table 3. Flow properties of aminated derivatives of natural gum

Polymers	Bulk Density	Tapped Density	The angle of repose (°)	Compressibility index (%)	Hausner's ratio	pH (1% w/v)
Tamarind gum	0.37	0.41	15.4	9.75	1.1	6.4
Aminated Tamarind gum	0.41	0.55	18.2	25.45	1.34	7
Xanthan gum	0.38	0.45	16.7	15.55	1.18	7.6
Aminated xanthan gum	0.43	0.52	19.1	17.3	1.2	8.2

*All value is mean of triplicate

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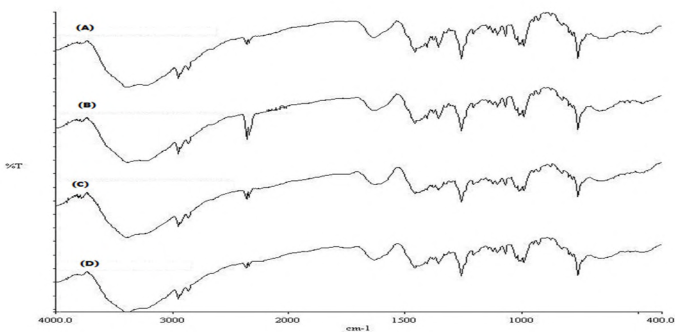

Fig. 1. ATR-FTIR of drug and prepared aminated derivatives **(a) LSP, (b) LSP + ATG, (c) LSP + AXG, (D) Formulation (AXG 3)*

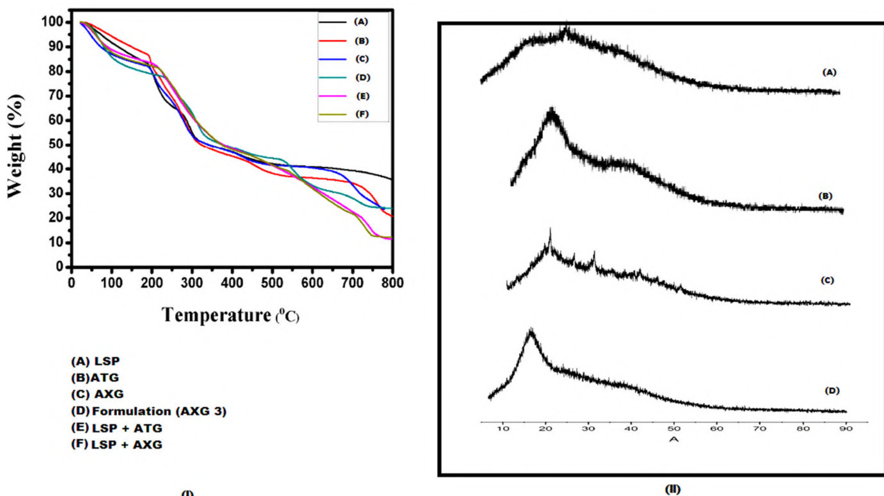

Fig. 2. DSC (I) and XRD (II) of drug and prepared aminated derivatives, *(A) LSP, (B) ATG, (C) AXG, (D) LSP+ ATG, (E) LSP+ ATG, (F)

$$C_{ss} = \frac{33 \times 50}{42 \times 10} = 7.85\text{mg/lit}$$

Now,

$$IRD = \frac{Vd \times Css}{F} = \frac{34 \times 07.85}{33} = 8.08\text{mg}{\sim}50\text{mg}$$

The total dose required to achieve sustained drug delivery from formulation throughout 12 h can be calculated using the following formula,

$$Total\ dose \div MD = 8.08\,[1 + (0.693 \times 2)] = 19.57 \cong 100mg$$

Where $t_{1/2}$= half-life (2 h); t = time up to which sustained action is needed (12 h). Therefore, from a total of 100 mg which was fixed as a sustained release dose of formulation, 19.57 mg (19.57 %) which was considered an immediate release fraction of the drug should release within 1 h to get the therapeutic effect and the remaining 80.83 mg of drug fraction for another 11h at a rate of 19.57 % at each hour. Thus, the theoretical drug release profile can be predicted using the above considerations as shown in figure 3 and is used to compare with the release profile of experimental batches.

**CR- Cumulative drug release*

Release behaviour of synthesized aminated derivative of natural gum

A matrix tablet of Losartan potassium was prepared by the wet granulation method. Five batches were prepared, consists T1 as a without aminated derivative of natural gum containing carbopol 934 (100 mg). Simultaneously T2, T3, T4 and T5 consist of ATG (30-40 mg), and AXG (30-40 mg) in various concentrations. Powder blends were prepared and subjected to further evaluation. Finally, tablets were compressed using a round flat-faced 12 mm die and punch. The compression force of 5 tones was kept constant for all formulations up to 15 s dwell time.

Evaluation of granules

All formulation shows a compressibility index between 7.7±1.4 to 9.8±1.8. As per the compressibility index, all formulation batches show excellent flow (Table no. 4). Hausner's ratio of granules of trial batches was found to e less than 1.5. All granules of trial batches show excellent flow properties as per Hausner's ratio. The angle of repose of granules in trial batches shows good flow property.

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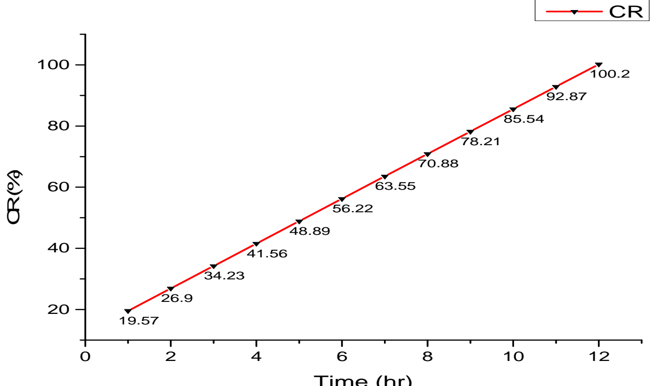

Fig. 3. Theoretical drug release profile of LSP for sustain release dose **CR- Cumulative drug release*

Table 4. Pre-compression parameter study of trial batches

Batch[a]	Bulk density	Tapped density	Compressibility index	Hausner's ratio	Angle of repose
T1	15±0.12	16±0.16	8.1±0.55	1.1±0.0065	28±0.65
T2	15±0.13	16±0.11	7.7±1.4	1.1±0.016	29±0.38
T3	15±0.17	17±0.19	9.8±1.8	1.1±0.022	28±0.37
T4	15±0.13	16±0.19	8±1.8	1.1±0.022	29±0.38
T5	15±0.13	16±0.19	8±1.8	1.1±0.022	29±0.38

**All Values are expressed as mean ± SD (n=3), p value<0.0001, p<0.05 considered statistically significant; [a] preliminary trial batches*

Evaluation of matrix tablet

The thickness and diameter of the tablet were measured by a Vernier calliper. The thickness and diameter of the tablet were found to be in an acceptable range. The hardness of the tablet was measured using a Monsanto hardness tester. The hardness of the tablet was found to be in the range of 4.1±0.058 to 4.8±0.1 (Table no. 5).

In vitro drug release study

In vitro drug release study of release modulating hydrophilic matrix system of losartan potassium was described in table no.6.

Batch T1 contain carbopol 934 (100 mg) and show 71% drug release in 12 hr, this is not acceptable for sustained release delivery of losartan potassium. To overcome the concentration of carbopol 934 in the matrix system, aminated polymers are taken in different ratios. The concentration of carbopol 934 decreases with the concentration of aminated polymers increases. Carbopol 934: ATG / AXG (ratio 3:1) shows 80 % and 86 % drug release in 12 hr respectively, the release profile of T2 and T3 does obey the release profile of LSP for sustain release dose shown in figure 4.

Carbopol 934: ATG/ AXG (ratio 1:1) show 99 % and 100 % drug release respectively. The release profile of T4 and T5 was very different from TDRP. Based on in vitro drug release study of trial batches we concluded that the required drug release profile of formulation is achieved by decreasing concentrations of carbopol 934 i.e. 60 to 70 mg. Concentrations of ATG and AXG extend the release of LSP for 12 hr shown in figure 5.

**T1, T2, T3 and T4 are the formulation code of preliminary trial batches with various concentrations as per table 1; CDR- Cumulative drug release in percentage*

**T1, T2, T3 and T4 are the formulation code of preliminary trial batches with various concentrations as per table 1.*

Preparation of Losartan potassium release modulating matrix tablet

A release modulating matrix tablet of LSP was prepared by the wet granulation method. The concentration of ATG and AXG was selected based on preliminary trial bathes. As per the experimental design, powder blends were prepared and subjected to further evaluation. Finally, tablets were compressed using a round flat-faced 12 mm die and punch. The compression force of 5 tones was kept constant for all formulations up to 15 s dwell time.

Evaluation of granules

Evaluation of granules is used to determine of flow property of granules. In the development of tablet formulation flow property of granules also affect the post-compression parameter of the tablet. The flow property of the tablet is described with various evaluation parameters shown in table no. 8. E.g. Bulk density, tapped density, angle of repose, Hausner's ratio and Carr's index.

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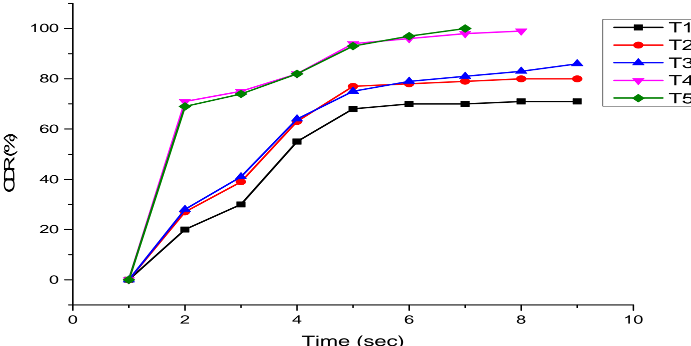

Fig. 4 *In vitro* drug release profile of preliminary trial batches. Drug release extending is directly propositional to the concentration of aminated derivative of natural polymers. **T1, T2, T3 and T4 are the formulation code of preliminary trial batches with various concentrations as per table 1; CDR- Cumulative drug release in percentage*

Table 5 . Post-compression parameter study of trial batches

Batch[a]	Thickness (mm)	diameter (mm)	Hardness (kg/cm2)	Friability (%)	Weight variation	Uniformity of drug content
T1	3.1±0.058	12	4.8±0.1	0.54±0.095	398.95	99±0.36
T2	3.1±0.0	12	4.3±0.15	0.66±0.21	398.6	99±0.36
T3	3.20±0.058	12	4.3±0.058	0.71±0.068	398.95	99±0.45
T4	3.1±0.058	12	4.1±0.058	0.6±0.057	396.15	99±0.47
T5	3.1±0.058	12	4.1±0.058	0.6±0.059	395.65	99±0.21

**All Values are expressed as mean ± SD (n=3), p value<0.0001, p<0.05 considered statistically significant; [a] preliminary trial batches*

Table 6. *In vitro* drug study of trial batches

Time	T1	T2	T3	T4	T5
0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
30	20±0.26	27±0.27	28±1.4	71±2.1	69±2.1
60	30±0.43	39±0.95	41±0.96	75±1.5	74±1.4
120	55±1	63±1	64±0.41	82±0.23	82±0.25
240	68±0.67	77±0.99	75±0.41	94±0.23	93±0.29
360	70±0.66	78±0.98	79±0.41	96±0.23	97±0.25
480	70±0.67	79±0.79	81±0.43	98±0.23	100±0.24
600	71±0.57	80±0.91	83±0.41	99±0.21	-
720	71±0.58	80±0.84	86±0.41	-	-

**All Values are expressed as mean ± SD (n=3), p value<0.0001, p<0.05 considered statistically significant.*

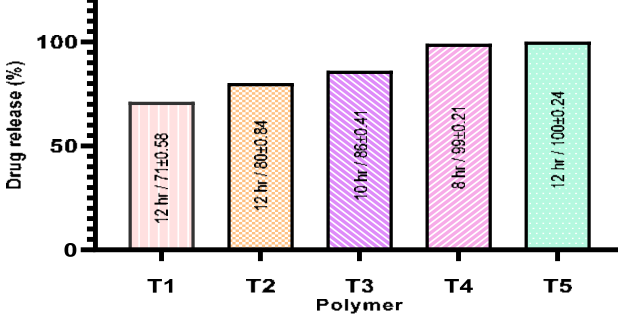

Fig. 5. Comparative study of polymer concentration on drug release of preliminary trial bathes *T1, T2, T3 and T4 are the formulation code of preliminary trial batches with various concentrations as per table 1.

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Table 7. Formulation table of development of LSP matrix tablet

Ingredient*	Batches					
	ATG1	ATG2	ATG3	AXG1	AXG2	AXG3
LSP	100	100	100	100	100	100
Carbopol 934	70	65	60	70	65	60
ATG	30	35	40	-	-	-
AXG	-	-	-	30	35	40
Magnesium Stearate	10	10	10	10	10	10
Microcrystalline cellulose	190	190	190	190	190	190
The total weight (mg)	400	400	400	400	400	400

**All values expressed in milligram (mg).*

Bulk density and tapped density of prepared granules were found to be 15±0.075 - 15±0.18 and 17±0.12 - 17±0.2 respectively. The per cent compressibility index of granules was found in the excellent range. Hausner's ratio of granules of all batches was found to be less than 1.25, thus all granules show excellent flow properties. Granules of all batches show good flow properties as per angle of repose between 28±0.38 - 29±1.4.

Evaluation of release modulating Losartan potassium matrix tablet

The thickness and diameter of prepared formulations were measured by Vernier calliper. The thickness of the prepared formulation was found to be in the range of 3.1 to 3.2. All formulation show uniform diameters i.e. 12 mm. Hardness of all formulations achieved about the same. Friability of prepared release modulating hydrophilic matrix tablet shows in the range between 0.35±0.11 to 0.36±0.19. The weight variation of formulated tablets was found to be between 396.6 to 399.85, and it is acceptable (Table no. 9).

In vitro drug release study

In vitro drug release study of release modulating hydrophilic matrix tablet was described in table no. 10. In vitro drug release profile of release modulating hydrophilic matrix system was studied in 0.1N HCl for 2 hr and in pH 6.8 PBS for next 10 hr. Batch AXG 3 maximum similarity with predicted TDRP of LSP for sustain release dose. The lowest concentration of carbopol 934(60 mg) and the highest concentration of AXG (40 mg) show extended drug release i.e. about 100 % in 12 hr which is acceptable for sustained delivery of LSP. If the concentration of carbopol 934 was decreased, the rate of drug release was increased for an extended period.

*Cdr- Cumulative Drug Release

Swelling studies

The swelling and erosion functioning of prepared matrix tablet in 0.1N HCl and Phosphate Buffer Solution, pH 6.8, as a function of time, is shown in figure 7. It can be noticed that the hydrophilic matrix tablets undergo both swelling and erosion at the same time. Batch AXG 3 are subjected to swelling and erosion study. Because AXG 3 show a similar dissolution profile with TDRP of LSP for sustained release. Batch AXG 3 show less swelling after up to 2 hr and shows more swelling after 4 hr in both media. Batch AXG 3 contains 40 mg aminated xanthan gum which shows good swelling properties in both mediums. It helps in the development of sustained release formulation of losartan potassium.

After 1 hour, Batch AXG 3 swelled to its full extent, which may be attributed to LSP's first burst release. According to the matrix tablet' simultaneous swelling and erosion, such hydrophilic systems can produce constant release.

Optimization

Optimization of formulations was carried out by comparing the drug release profile of formulation with TDRP of LSP for sustained release dose. The theoretical drug release profile was calculated for a 100 mg dose of LSP for one day. TDRP show 19.57 % drug release at 1 hr was to be significant for the initial burst release of LSP. After the initial burst release, drug release was increased by 7.33 % / hr up to the 12th hr.

AXG 3 shows about similar drug release profile concerning predicted TDRP of LSP for sustained release. ATG 3 shows about 100±0.024 % drug release up to 12th hr, but as per predicted TDRP there are about 100.2 % drug release was required at 12th hr. AXG 3 exhibits greater similarity with TDRP shown in figure 8. So, thus AXG 3 was selected as the best formulation for oral sustain drug delivery of LSP.

Discussion

Reductive amination of native Tamarind and Xanthan was synthesized successfully by using ethylene diamine and sodium borohydride as an aminating agent and reducing agent respectively. The amination reaction was successfully implemented after some of the hydroxyl groups on natural gums were deoxidized. The resulting products showed a great quantity of primary amine group and secondary amine group which can be used as swelling agents. Aminated Tamarind gum shows poor flow properties as compared to aminated Xanthan gum and its native form in the performed investigation. But both derivatized moieties are compatible with a model drug used in the formulation of sustain release matrix tablet. Amination of natural polymers affects the

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Table 8. Pre-compression parameter study of release modulating matrix of LP

Batch	Bulk density	Tapped density	Compressibility index	Hausner's ratio	Angle of repose
ATG1	15±0.14	17±0.2	12±0.44	1.1±0.0056	28±0.38
ATG2	15±0.075	17±0.16	11±0.42	1.1±0.0053	29±0.65
ATG3	15±0.15	17±0.19	11±0.62	1.1±0.0079	28±0.38
AXG1	15±0.12	17±0.15	12±1.3	1.1±0.017	29±1.4
AXG2	15±0.18	17±0.12	11±0.89	1.1±0.011	29±0.38
AXG3	15±0.12	17±0.14	12±1.0	1.1±0.013	29±1.4

** All Values are expressed as mean ± SD (n=3), p value<0.0001, p<0.05 considered statistically significant.*

Table 9. Post-compression parameter study of release modulating matrix of LP

Batch	Thickness (mm)	diameter (mm)	Hardness (kg/cm2)	Friability (%)	Weight variation	Uniformity of drug content
ATG 1	3.2±0.0	12	4.2±0.1	0.36±0.19	396.6	99±0.36
ATG 2	3.1±0.058	12	4.2±0.1	0.35±0.11	398.55	99±0.37
ATG 3	3.1±0.058	12	4.3±0.15	0.31±0.17	399	98±0.55
AXG 1	3.2±0.058	12	4.3±0.15	0.31±0.17	399.85	98±0.36
AXG 2	3.1±0.058	12	4.3±0.058	0.34±0.15	399.8	99±0.27
AXG 3	3.1±0.058	12	4.2±0.058	0.33±0.14	398.55	99±0.45

**All Values are expressed as mean ± SD (n=3), p value<0.0001, p<0.05 considered statistically significant.*

Table 10. *In vitro* drug release study of release modulating matrix of LP

Time	ATG1	ATG2	ATG3	AXG1	AXG2	AXG3
0 min	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
30 min	29±0.72	31±0.012	15±0.046	29±0.14	20±0.016	15±0.32
1 hr	42±0.13	42±0.51	19±0.095	42±0.56	36±0.64	22±0.38
2 hr	65±0.83	67±0.014	44±0.045	70±0.17	75±0.36	35±0.24
4 hr	80±0.62	85±0.23	58±0.076	81±0.35	86±0.045	51±0.31
6 hr	82±0.77	86±0.025	71±3.3	85±0.56	90±0.084	64±0.38
8 hr	84±0.47	88±0.045	87±0.4	88±0.42	92±0.74	79±0.24
10 hr	86±0.81	89±0.0124	92±0.95	89±0.71	94±0.086	91±0.24
12 hr	86±0.83	91±0.23	97±0.45	92±0.76	96±0.076	100±0.24

**All Values are expressed as mean ± SD (n=3), p value<0.0001, p<0.05 considered statistically significant.*

Formulation (AXG 3)

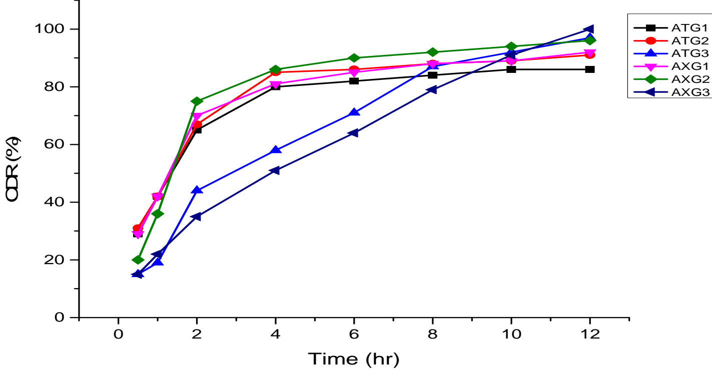

Fig. 6. Dissolution Profile prepared formulation up to 12th hours. AXG3 exhibit minimum burst release up to 2nd hours with a high concentration of AXG. Increasing the concentrations of aminated derivatives of natural polymer are extend the drug release up to 12th hours. **CDR- Cumulative drug release*

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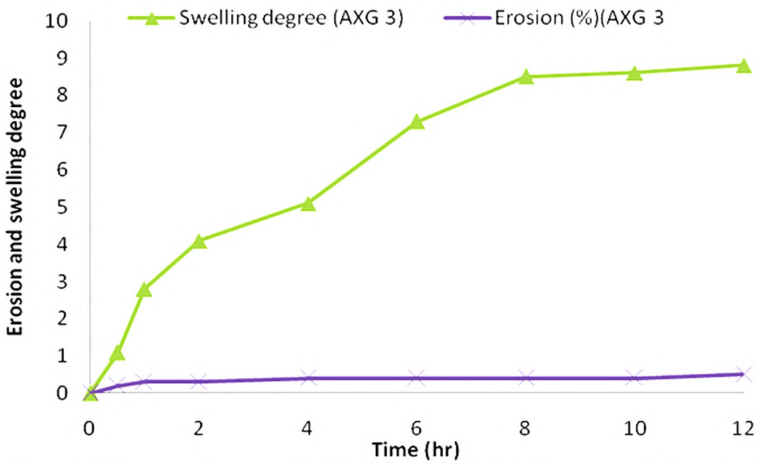

Fig. 7. Erosion and swelling behaviour of optimized formulation i.e. AXG 3 shows good swelling compared with other formulations.

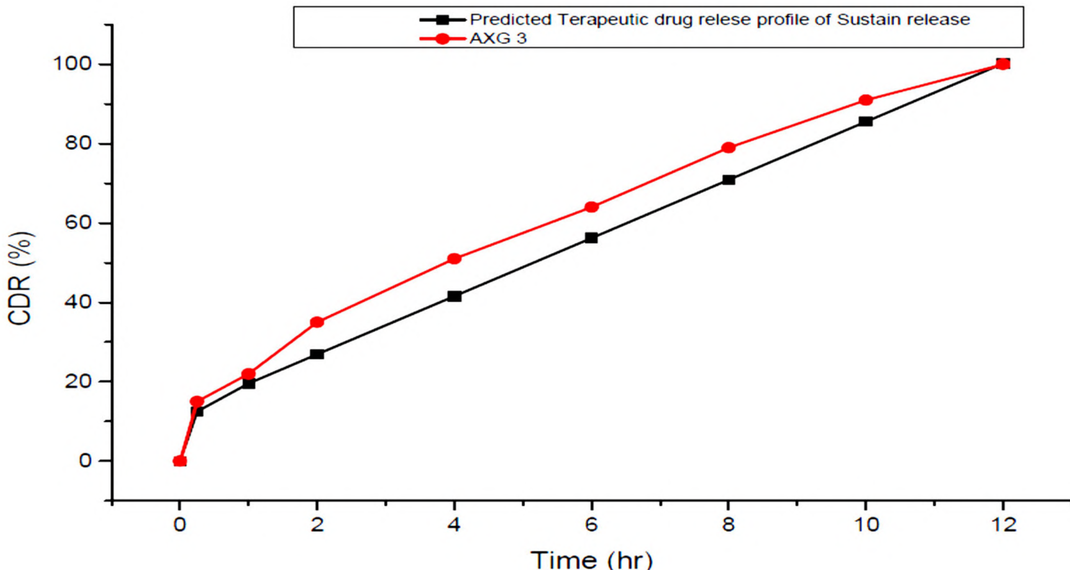

Fig. 8. Dissolution profile of optimized batch AXG 3 compared with predicted TDRP of LSP for sustain release dose, AXG 3 shows maximum similarity with predicted TDRP. **CDR- Cumulative drug release in percentage*

crystallinity of their native form. The amino derivative of natural gum shows amorphous behaviour more than its original form. Physical form-changing properties of native gum after amination exhibit good binding and long-lasting bio-adhesion strength. In the present investigation sustain release matrix tablet formulation was developed using derivatized ATG and AXG as a swelling agent and both derivatized polymers show good adhesive properties. In prepared formulation batches concentration of Carbopol 934 decreased as the concentration of aminated polymers was increased. Hydrophilic matrix tablets of LSP with ATG and AXG were prepared and optimized using a theoretical drug release profile of LSP for sustained release dose. The dissolution profile of optimized formulation AXG 3 was compared with the calculated TDRP of LSP for sustained release dose. On basis of the dissolution profile of optimized formulation, we observed that batch AXG 3 shows maximum similarity with TDRP of LSP for sustain release.

Overall, the study indicates that aminated tamarind gum and aminated Xanthan gum can be promising candidates for designing sustain-release bioadhesive drug delivery systems. Because developed matrix tablets absorb more amount of water and form a sponge-like structure. In this case, the spongy nature of modified gum with the aqueous medium can be applicable for long-lasting bioadhesive formulation. Modified amino derivatives of natural polymers can be promoted excellent mechanical strength to bioadhesive drug delivery for sustained release to obtain constant release from formulation. The formulated smart crosslinked ATG and AXG demonstrated acceptable biodegradability

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with no cellular toxicity, suggesting their applicability as pH-sensitive oral drug carriers.

Conclusion

Tamarind gum and Xanthan gum have been functionalized with the amino group. ATG and AXG at very high concentrations (i.e. 40 mg) in prepared matrix form extend the release rate and maintain the constant therapeutic concentration of the drug in the bloodstream. The bonding of the amino group to the ATG and AXG polymers was confirmed by FTIR spectra. The modified amino derivatives of Tamarind and xanthan have the same solubility as native forms. The native form of gum showed a broad melting point around 75-78°C whereas modified amino derivatives were at 105-115°C. In addition, modified amino derivatives exhibited good thermal properties. This modified derivative has potential applications in the medical and bio-tronics field because it possesses biocompatibility and strong binding behaviour with a very useful application.

Trial batches of LSP matrix tablets give an idea about formulation development. A high concentration of carbopol 934 (100 mg) shows unnecessary drug release behaviour as compared to TDRP of LSP for sustained release dose. Thus, synthesized aminated derivatives of TG and XG are subjected to formulation to minimize the concentration of carbopol 934. Carbopol 934: ATG / AXG is incorporated in the formulation in different ratios i.e. 3:1 and 1:1. Here low concentration of aminated derivatives in matrix tablet of LP show about 70-72 % drug release for 12 hr and high concentration of aminated derivatives show about 100 % drug release in 8-10 hr. Based on trial batches we concluded that, for obtaining similar TDRP of prepared formulation, the concentration of carbopol 934 (70-60 mg), ATG (30-40 mg) and AXG (30-40 mg) was selected.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgement

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Creation and Development of Promethazine (PT) Fast Dissolving Tablet Using Quality by Design Methodology

Indrajit Kadam[1], Smita Borkar[1], Vishal Yadav[1], Prakash Jadhav[2], Ashish Thorat[2], Vinay Gaikwad[3*]
[1]Assistant Professor, Department of Pharmaceutics, Arvind Gavali College of Pharmacy, Satara, Maharashtra, India
[2] Assistant Professor, Department of Pharmaceutics, YSPM's Yashoda Technical Campus, Satara, Maharashtra, India
[3] Assistant Professor, Department of Pharmaceutics, S.P.M.'s College of Pharmacy (Poly), Akluj, Maharashtra, India

[*]Corresponding Author: vinaygaikwadcopa@gmail.com

ABSTRACT

Patients continue to choose the oral route for medicine delivery over other available dosage forms. According to the research, over half of patients will eventually favor oral disintegrating tablets (ODTs) over alternative solid oral dose forms. The oral route is the most favored for administering medication since it has several advantages over other routes. Patients get a variety of pharmacological therapeutic substances used in ODTs to spread their effects throughout the body. Because of the extremely high risk of aspiration and difficulty swallowing, ODTs are recommended for asthma patients. In addition to the restrictions listed above, it is ideal when the patient is on the go or has limited access to water. For the creation of ODTs, several patented and unpatented preparation techniques or technologies are available. Promethazine (PT), a first-generation H1 receptor antagonist, is a medication used to treat motion sickness that is also used as an antihistamine and antiemetic. Due to PT's 2.2-hour half-life and first-pass hepatic degradation, its 88 percent oral bioavailability has been decreased to 27%. As a result, efforts have been undertaken to create tablets that dissolve quickly using the direct compression technique. The tablets were created using the quality by design (QbD) method to successfully transfer technology. Nowadays, risk management for successful QbD has become essential for product approval as the FDA evaluates the execution and effectiveness of the procedure, formulation design as detailed in the application, and QbD.

Keywords- Formulation design technology, H1 receptor antagonist, Oral disintegrating tablets, Promethazine, Quality by design approach (QbD)

INTRODUCTION

Patients continue to choose the oral route for medicine delivery over other available dosage forms. The research assumes that over 50% of patients will favor ODTs over other solid oral dosage forms in the future. Because it has several advantages over other routes, taking medication orally is the preferred method [1]. Due to the extremely high risk of aspiration and difficulty swallowing, ODTs are recommended for asthma patients [1, 2]. In addition to the restrictions described above, it is desirable when the patient is on the go or has limited access to water [2]. It is recommended since it is simple and accessible to a variety of patients.

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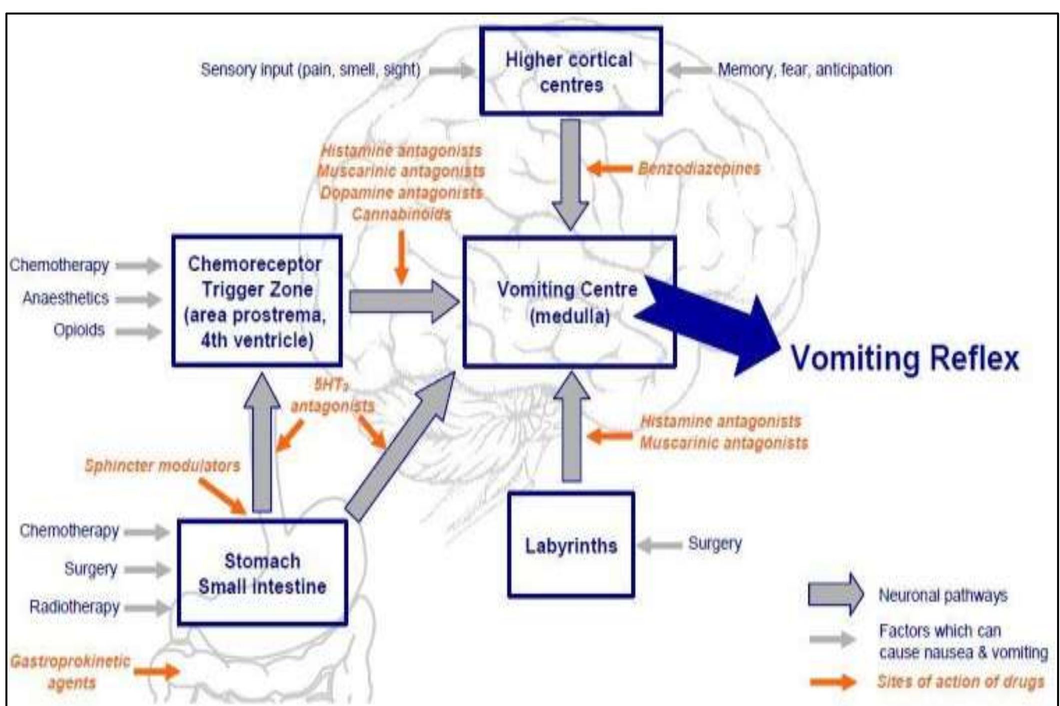

Figure 1: *Medication for nausea and vomiting.*

For the creation of ODTs, several patented and unpatented preparation techniques or technologies are available (Fig. 1). In addition to more traditional methods like tablet molding, direct compression, mass extrusion, spray drying, sublimation, and cotton candy process, patent-protected methods like Zydis technology, Orasolv technology, Durasolv technology, Wowtab technology, Flash tab technology, Flashdose technology are used. These strategies are shown in Table 1.

Table 1: *Tablets that dissolve in the mouth using patented technology.*

Sr. No.	Techniques	Involved Molecule	Process	Firm
1	WOWTAB®	Famotidine	Direct compression	Yamanouchi Pharma Technologies, 1050 Arastradero Road, Palo Alto, CA, USA
2	ORASOLV®	Paracetamol	Direct compression	Cima Labs, Inc., 10000 Valley Hill Road, Eden Prairies, MN, USA
3	DURASOLV®	Zolmitriptan	Direct compression	Cima Labs, Inc., 10000 Valley Hill Road, Eden Prairies, MN, USA
4	FLASHTAB®	Ibuprofen	Direct compression	Prographarm, haueauneuf- En-Thymeraia, France
5	LYOC®	Phlorglucinol hydrate	Lyophilization	Farmalyoc, 5AV Charles Marting, Maisons- Alfort, France
6	QUICKSOLV®	Risperidone	Lyophilization	Janssen Pharmaceutica, 1125 Trenton-Harbourton Road, Titusville, NJ, USA
7	ZYDIS®	Loratidine	Lyophilization	R. P. Scherer, Frankland Road, Swindon, UK
8	FLASHDOSE®	Tramodol hydrochloride	Cotton Candy Process	Fuisz Technologies, 14555 Avion At Lakeside, Chantilly, VA, USA

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As the size of the SPH microparticle reduced, it was discovered that the micro particle's tensile strength was very low or even decreased. The produced ketoprofen-loaded FDTs' tensile strength and period of disintegration were both significantly influenced by the size of the SPH microparticle [4]. However, in certain instances, it was discovered that when the size of the microparticle was reduced, the tensile strength of the produced FDTs rose. The microparticle, which ranged in size from 75 to 106 µm, was found to disintegrate in less than a minute. It was recommended that the ideal microparticle size should fall between 75 and 106 µm. It has been recommended to employ single disintegrants or to utilize a mixture of disintegrants in a patent that has been published [5].

It can also be made better by adding more insoluble inorganic excipients and calcium salts such as dibasic calcium phosphate [6]. Components including flavoring agents, compressible sugars, binders, sweeteners, and surfactants are considerably soluble. The time it takes for the tablets to dissolve depends on the weight ratio between the water-soluble and water-insoluble excipients. Organic filler or insoluble inorganic salt are examples of excipients. As the proportion of insoluble components decreases, it has been claimed that the disintegration time would increase extremely quickly. The proportion of insoluble inorganic components employed in conjunction with disintegrants, they observed, had an impact on how easily manufactured FDTs disintegrated. The ideal excipients for this are those that are naturally water soluble [6, 7].

A key property of oral medication delivery systems is their palatability. In addition to maintaining the mechanical strength needed for tablet manufacture and distribution, it also disintegrates at a quick rate. It causes ODTs to continue to expand, which allows them to disintegrate at a very high and quick rate [8, 9]. Different kinds of sweetening and flavoring additives are needed to cover up the drug's harsh taste. It depends on how well-liked or effective ODTs are, which is further decided by the intended patients. ODTs have a quicker or faster beginning of action as a result of their diffusion in the upper section of the GIT [8]. One of the greatest coating methods for effectively disguising the bad taste of APIs is known as Coacervation. It has been claimed that utilizing monoglycerides is a unique way to disguise the taste of macrolides. In addition to masking the flavor, it accelerates the pace at which the APIs dissolve and disintegrate. The choice of ingredients used to make ODTs relies on how unpleasant or bitter the medications taste. Various techniques, including solvent evaporation and solvent extraction, are employed to improve the flavor of drugs. For the microencapsulation of medications, several pH-sensitive acrylic polymers, including Eudragit L-55, Eudragit E, and Eudragit RL, are used. These polymers are used to mask the disagreeable taste of the medication. The flavor of the medication is covered up using the microencapsulation technique. Several taste masking techniques are used to hide or disguise the unpleasant, bitter, or horrible taste of the medication [10].

The failure of therapy occurs when medications with limited bioavailability are partially lost through vomiting. Generally, nausea precedes vomiting, making it challenging to deliver medication with a glass of water. As a result, it is preferable to administer medications as fast-dissolving pills. The retention and absorption of the medicine are also impacted by dizziness, pregnancy, migraines, physiological processes including reduced stomach emptying, and other gastric problems [11, 12]. As a result, maintaining the oral dosage is necessary for absorption to prevent vomiting. The diaphragm must compress downward to violently vomit. As a result, there is poor bioavailability, which does not reduce the rate. The sphincter remains open when the abdominal muscles contract against a relaxed stomach. Emesis is only a symptom of changed physiological processes; it is not an illness. After oral dosing, this antiemetic has significant first-pass gastrointestinal activity [13].

MATERIALS AND METHODS

A first-generation antihistamine called promethazine (PT) is used to treat allergies, motion sickness, and nausea. Promethazine sulfoxide is the main product of PT metabolism, with desmethylpromethazine and a hydroxy metabolite coming in second and third place. Croscarmellose sodium, Lactose monohydrate (Pharmatose 200), Magnesium Stearate, Microcrystalline Cellulose, and PT for this current work were supplied by Abbott Pharma, India, DFE Pharma, India, Peter Greven, India, and Merck, India, respectively.

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Before formulating API, preformulation tests were carried out to determine the properties, identification, and stability of the medication in the formulation [14]. To determine the purity and describe the likely structural alteration of the drug sample, infrared spectroscopy was used to evaluate the PT sample. With the use of an infrared spectrophotometer and Fourier transforms, sample spectra were performed between 4000 and 400 cm^{-1}. To reduce peak interference caused by functional groups contained in KBr, a blank KBR spectrum was conducted. The drug sample and blank KBR were combined in a 1:9 ratio before being poured into the cavity. The change in the appearance of these mixes was examined physically. An early risk reduction method is a medication and excipient compatibility study. For 15 to 20 minutes, potassium bromide (KBr) was allowed to activate in a hot air oven [15]. Excipients that could interact with the drug ingredient are not allowed to be used. An early risk reduction method is a medication and excipient compatibility study. Excipients that could interact with the drug ingredient are not allowed to be used. PT was triturated at a 1:1 ratio with each excipient separately. The samples were kept at 40°C and 75% RH for 4 weeks. To verify changes in appearance, these mixes were physically evaluated [16].

Formulation and Development of PT-FDT by QbD Approach

QTPP (Quality target product profile) (Quality target product profile) an outline of the qualities of a drug product's quality that should ideally be attained to assure the intended quality, taking into account the medication product's safety and efficacy. Avomine® (oral tablet), a chosen RLD (Reference Listed Drug), was used to identify QTPP for tablet formulation and development [17]. Table 2 provides a summary of the main QTPP constituents, including identification, mode of administration, dose, dosage form, dissolution, and disintegration.

Table 2: *QTPP of a tablet.*

QTTP Component	Focus	Explanation
Physical		
Route of administration	Oral	Requirement for pharmaceutical equivalence: same method of administration
Dosage form	Tablet	Requirement for pharmaceutical equivalence: identical dosage form
Dosage strength	25mg	Requirements for pharmaceutical equivalents: same strength
Therapeutic moiety release/delivery	Fast dissolving tablet	To fulfill label claims comparable to the references
QTTP Component	**Focus**	**Explanation**
Chemical		
Assay	Not less than 90% and not more than 110% of the labeled amount of PT	Required to maintain safety and efficacy.
Disintegration time	NMT 30 sec	The drug profile is important to achieving bioequivalence (BE).
Dissolution Profile: pH 6.8 USP Type II, Volume:900mL, Speed: 50 rpm, Time points(min): 5, 10, 15, 20, 30 and 45	NLT 80% (Q) labeled amount of PT dissolved in 15 minutes.	To achieve bioequivalence, the pharmacological profile is crucial (BE). A similar drug release profile with a reference product is sought after to guarantee bioequivalence since in vitro drug release serves as a proxy for in vivo performance.
Biological		
Intended use	Intended to treat people	Therapeutic equivalence requirement:

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	with non-valvular atrial fibrillation and lower their risk of stroke and systemic embolism.	Same indication.
Packaging and Storage		
Container closure system	The container closure mechanism was determined to be appropriate for this pharmaceutical medication.	Required to maintain tablet integrity throughout transportation and to reach the desired shelf-life.
Storage condition	Away from direct sunshine, in a cool, dry environment, and at room temperature.	Room temperature in a cool, dry location away from the sunlight.

Innovator Product Characterization

The RLD selected was Avomine®. The analysis of RLD was carried out. Product identification of RLD is given in Table 3.

Table 3: *Product identification of RLD Avomine®.*

Parameter	Description
Batch No.	31171043
Mfg. Date	SEP.2020
Exp. Date	AUG 2022
Label claim	Each tablet contains 25 mg of PT

Table 4: *PT-FDT CQAs (Critical Quality Attributes).*

Characteristics of an Effective Drug Product	Focus	Is it CQA?	Explanation
Disintegration time	similar to RLD	**YES**	To be comparable with the innovator Avomine ®
Dissolution	NLT 80% of the Labeled amount of Drug should dissolve in 15 min	**YES**	To achieve bioequivalence, the pharmacological profile is crucial (BE). Since in vitro drug release serves as a proxy for in vivo performance, a reference product's drug release profile should be the goal to verify bioequivalence.
Palatability	Sweet taste	**YES**	Patient compliance

Table 5: *Overview of the preliminary risk analysis.*

Low	An acceptable level of danger. No more research is required.
Medium	Risk acceptance It could be necessary to do further research to lower the danger.
High	Risk cannot be tolerated. To lower the danger, further research is required.

Table 6: *Initial risk analysis.*

Drug Product CQAs	Level of CCS	Level of Magnesium Stearate	Hardness
Disintegration time	High	Low	High
Dissolution	Low	Medium	High

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Identification of CQA's

CQAs was identified based on the effect of quality attribute safety, and efficacy of the formulation on a patient. Table 4 shows the CQAs for PT tablets.

Initial Risk Analysis

To find any possible interactions between the medicine, excipients, different unit activities, and key features, a preliminary risk assessment was conducted. The goal was to identify the damaging event, its origin, the likelihood that it will occur, its effect, and a method for detecting it [18].

DESIGN OF EXPERIMENT (DOE)

Excipient selections were made following preliminary tests using various ratios. The CQAs were influenced by the CCS level, magnesium stearate content, and tablet hardness. The optimization experiments were chosen from randomized blind batches (Table 5 & 6). The optimization investigations used a Box-Behnken design [19]. Researchers looked at how excipients affected CQAs for medicinal products at different concentrations. Magnesium stearate (B), CCS (A), and hardness (C) were chosen as independent variables (Table 7).

Table 7: *Coded levels for Box-Behnken design.*

Sr. No	Independent Variable	Level		
		-1	0	+1
1	Level of CCS (%)	4	5	6
2	Magnesium stearate (%)	0.5	1	1.5
3	Hardness (N)	50	100	150

Characterization of Tablet

Pre-Compression Characterization

Bulk Density

A compound's bulk density varies greatly depending on the method of crystallization, grinding, or formulation. It is determined by using a large funnel to pour the pre-sieved powder into a graduated cylinder, then determining the extent and weight using the formula in equation 1.

$$\text{Bulk density} = \frac{\text{weight of powder}}{\text{bulk volume of powder}} \quad (1)$$

Tapped Density

A graded tape is used to calculate the tapped density, which may then be estimated using equation 2.

$$\text{Tapped density} = \frac{\text{weight of powder}}{\text{tapped volume of powder}} \quad (2)$$

The volume of the powder bed must achieve a minimum volume in a cylinder with a specified powder mass and a mechanical extraction device that runs for a predetermined number of taps (10.500.1250). It is possible to gauge thread density.

Carr's Index

Bulk density and tapped density measurements are used to calculate Carr's index. Carr's index is determined using the following equation (3).

$$CI = \frac{(\text{Dt}-\text{Db})}{\text{Dt}} \text{x}100 \quad (3)$$

Where, D_t = Tapped density and D_b = Bulk density

Hausner's Ratio

The flowability and packing capacity are shown by Hausner's ratio. The capacity of materials to flow and pack is satisfactory when Hausner's ratio is near 1. The following equation 4 was used to compute Hausner's ratio.

$$\text{Hausner's ratio} = \frac{\text{Dt}}{\text{Db}} \quad (4)$$

Where, D_t = Tapped density and D_b = Bulk density

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Table 8: *Acceptable limits for flow properties.*

Parameter of Compressibility	Flow Nature	Hausner's Ratio
1 – 10	Excellent	1.00 – 1.11
11 – 15	Good	1.12 – 1.18
16 – 20	Fair	1.19 – 1.25
21 – 25	Passable	1.26 – 1.34
26 – 31	Poor	1.35 – 1.45
32 – 37	Very Poor	1.46 – 1.59
> 38	Very very poor	> 1.60

Tablet Evaluation

Weight Variation

It is ideal for the weight of each pill in a batch to be consistent. If there is any weight variance, it should be within the permitted ranges (Table 8). Each trial's 20 core pills were weighed on a calibrated scale to check for weight fluctuation and compare the results to the acceptable thresholds [20] (Table 9).

Table 9: *USP's guidelines for acceptable variations in tablet weight.*

The Average Tablets Weight (mg)	Maximum Percentage Difference Permitted
130 or less	±10
130-324	±7.5
More than 324	±5

Hardness and Thickness

The only dimension linked to the procedure is the tablet's thickness and hardness. The Erweka Hardness Tester was used to determine the tablets' hardness and thickness.

Disintegration Time

The disintegration time of 6 core tablets of each trial was checked and compared with the limit mentioned in USP.

In-Vitro Dissolution Studies

With sodium phosphate buffer pH 6.8, 50 rpm, and USP type II equipment, in vitro release tests of produced PT-FDT were carried out at 5, 10, 15, 20, 30, and 45 min (ELECTROLAB). To keep the sink condition, an aliquot (5 ml) was removed and replaced with a new medium. With the use of a double-beam UV visible spectrophotometer and a dissolving media used as a blank, filtered samples were properly diluted before being examined at 249 nm. Using a calibration curve created from a reference standard, the quantity of drug contained in the samples was determined. RLD and the drug product were contrasted to evaluate the drug release profile and physicochemical characteristics of the drug product [21].

Updated Risk

Based on the CQAs and initial risk assessments for CMA's the above risk for DOE will be updated. Further control strategy will be developed for the product followed by continuous product development [22].

Design Space

It has a huge selection of verified process parameters that guarantee quality. The alternative draws attention to the intricate relationships between input variables and consciously makes a connection between the established hierarchy of a design space and the DoE's behavior, which is mostly made up of interactions between input variables. A design space may be built for one unit operation, a few unit operations, or the full method [23].

Control Strategy

The range of recognized and proven process parameters used for quality control makes up the design space. You may set up a design room for the whole process, a few units, or one single unit.

Stability Study

According to ICH recommendations, the stability study was conducted on the improved

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formulation at 40°C and 75% RH for three months. A dissolving study was conducted to test the tablets [24].

RESULTS AND DISCUSSION

Infrared spectroscopy was used to investigate the PT sample to determine its purity and if any, any structural modifications. The material was examined between 4000 and 400 cm-1. The FTIR spectrum displayed fundamental peaks in the functional group-corresponding region. The presence of primary peaks in the spectrum proves that the sample used for testing was the PT shown in Fig. 2. The 10 g/ml solution's UV spectra were captured between 400 and 200 nm. At 249 nm, the greatest absorption was discovered. The absorbance values were measured using a double-beam UV spectrophotometer at various doses (Fig. 3). Fig. 4 shows a graph of absorbance vs. concentration. These graphs, which follow Beer-law, and Lambert's were linear in the concentration range of 2–25 µg/ml.

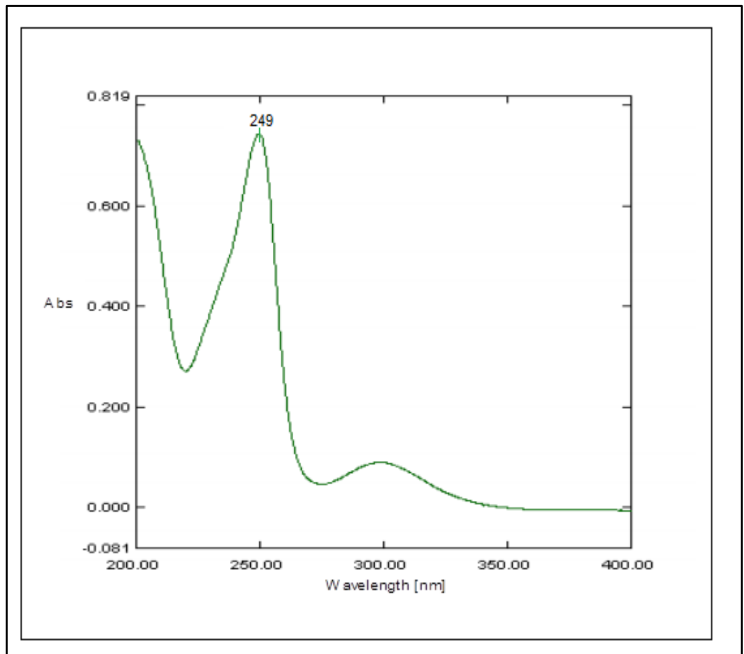

Figure 2: *FTIR spectra of PT.*

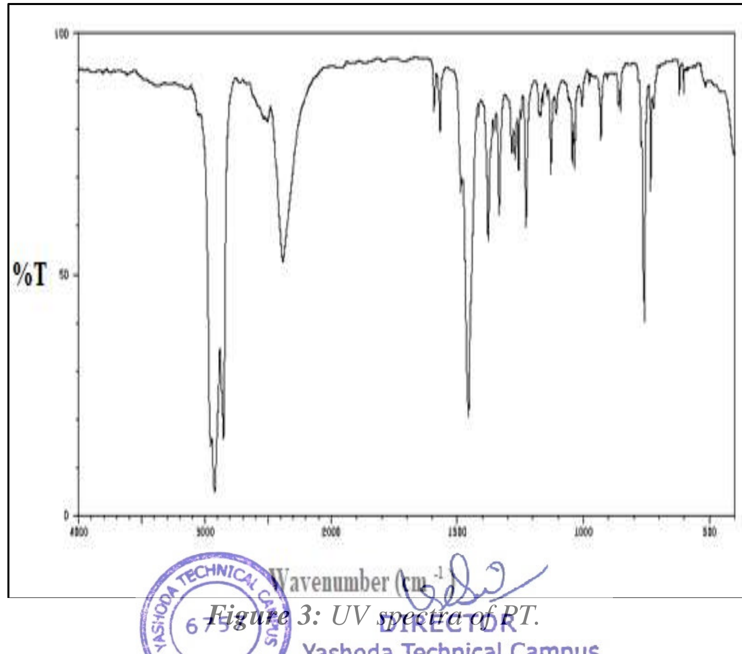

Figure 3: *UV spectra of PT.*

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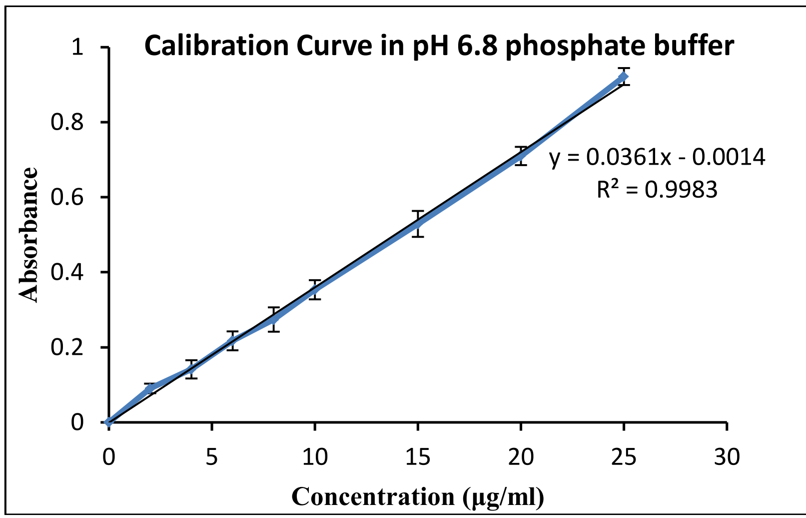

Figure 4: *Calibration curve of PT in pH 6.8 phosphate buffer.*

The physical examination of drug-excipient compatibility showed no change in the appearance of the drug with all the excipients in dry form as represented in Table 10. Thus all the selected excipients were compatible with the drug.

Table 10: *Drug and excipient compatibility study.*

Sr. No.	Sample	Initial Assessment	40°C/75%RH and After (4 weeks)
1	PT	White to off-white powder	White to off-white powder
2	PT+ Lactose monohydrate	White to off-white powder	White to off-white powder
3	PT+ CCS	White to off-white powder	White to off-white powder
4	PT+MCC	White to off-white powder	White to off-white powder
5	PT+ Mg stearate	White to off-white powder	White to off-white powder

Table 11: *According to the experiment's design, tablet batches (DoE).*

Run	Factor 1 A: Level of CCS (%)	Factor 2 B: Level of Mg Stearate (%)	Factor 3 C: Hardness (N)
1	6	1	50
2	4	1.5	100
3	4	0.5	100
4	5	1.5	50
5	5	0.5	50
6	4	1	50
7	6	0.5	100
8	6	1.5	100
9	5	0.5	150
10	5	1.5	150
11	6	1	150
12	4	1	150

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An examination was conducted before and after compression. The bulk density, tapped density, Carr's index, and Hausner's ratio of each trial batch were evaluated and contrasted with USP standards. All batches of powder were discovered to have fair to good flow qualities based on their micrometric properties. The tablets' disintegration time, weight fluctuation, hardness, thickness, and diameter were assessed, and the results were good compared to the goal characteristics (Table 11). The angles of repose, Carr's index, and Hausner's ratio have corresponding ranges that indicate good-excellent, good, and fair-good flow characteristics [25].

Studies on *in-vitro* dissolution: Each trial batch's PT-FDT dissolution was conducted individually under prescribed protocol, and drug release profiles were computed using a standard absorbance (Fig. 5). The batch with greatest r2 (0.9991) value and release kinetics that was closest to those of Avomine® was deemed to be the optimal batch. When compared to Avomine®, it too exhibited a similarity factor of 57 (f2=57).

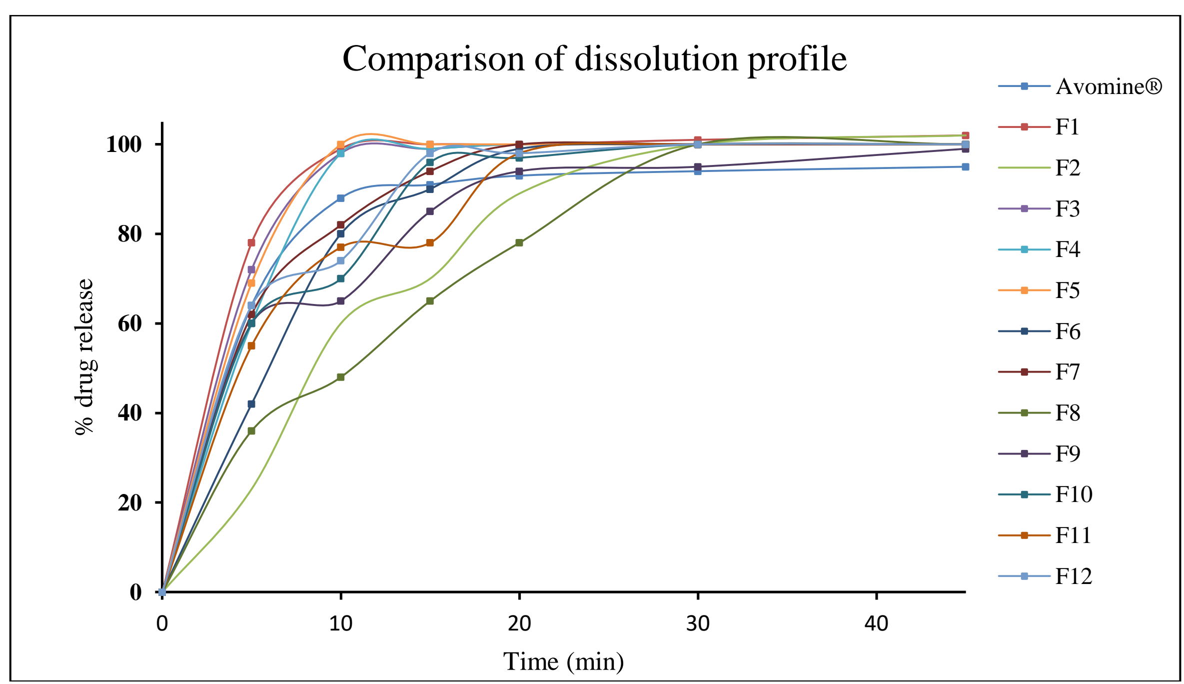

Figure 5: *Comparison of DoE batch in-vitro dissolution profiles.*

Assessment of Design of Experiment

The time it took for a tablet to completely dissolve was determined as Y1 (time taken to dissolve tablet entirely), and the dissolution rate (%DR) at the end of five minutes was computed as Y2 (dissolution rate). The %DR at 5 min was selected as the Y2 since there was a significant difference between the values that were gathered. Equations (1) and (2) were utilized in this design to determine the importance of each coefficient on main effects and interaction terms using p values and the appropriate polynomial model (2). The effect of the associated independent variable(s) is significant if the p-value is less than 0.05, which makes the corresponding coefficient more significant. A regression study of the rates of disintegration and dissolution's determination coefficients (R2) (0.99) showed that this design's strong correlation between the independent parameters accounts for 99% of the overall variations. A positive value for a response in the regression equation indicates a synergistic influence (direct link), whereas a negative value for a response indicates an inhibitory action (inverse relationship) on the regression model [26].

$$Y_1=8.8475-1.58875A-0.50875B+4.56C+0.5375AB+0.695AC+0.15BC-1.2675A^2-1.3775B^2 \quad (1)$$

$$Y_2=50.75+5A-1.75B-15C+2.25AB+10.75AC+0.25BC+7.5A^2+1.5B^2 \quad (2)$$

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The average disintegration time and drug release at 5 minutes (Y1 and Y2), as shown in eqs. (1) and (2), were 8.84 and 50.75, respectively. It was discovered that the answer Y1's positive coefficient of C was bigger than its negative coefficient of A, showing that the degree of CCS has an inverse relationship with DT whereas hardness has a direct relationship with DT. The lower values of the AB, BC, and AC coefficients suggest that the interaction between the A, B, and C variables may have a less significant effect on the disintegration time. Greater influence on drug release at 5 minutes is indicated by a greater coefficient of C in equation 2. The amount of medication released at 5 minutes decreased with tablet hardness, whereas reaction Y2 was directly correlated with CCS level. A positive AC coefficient in Y2 meant that CCS and hardness affected drug release behavior more than BC and AB. The effects of the amount of CCS, magnesium stearate, and toughness on Y1 disintegration time (Fig. 6 (a-c)) and Y2 (% release of drug at the end of 5 min) were determined using their respective 3D response surface plots. While 3D response surface graphs are more useful in understanding the main and interaction effects of the independent variables, contour plots emphasize comparison implications through a visual depiction of the response values [27].

The time needed to dissolve the pill reduced as the level of super disintegrant rose. The tablet's hardness had a significant impact on how quickly it disintegrated. Increased disintegration time was seen as the hardness increased. The disintegration was not significantly impacted by the amount of magnesium stearate since the variable was low risk. Hardness and the level of magnesium stearate both harmed the percentage of DR, whereas the level of CCS had a positive impact (Fig. 6 (d-f)) [28].

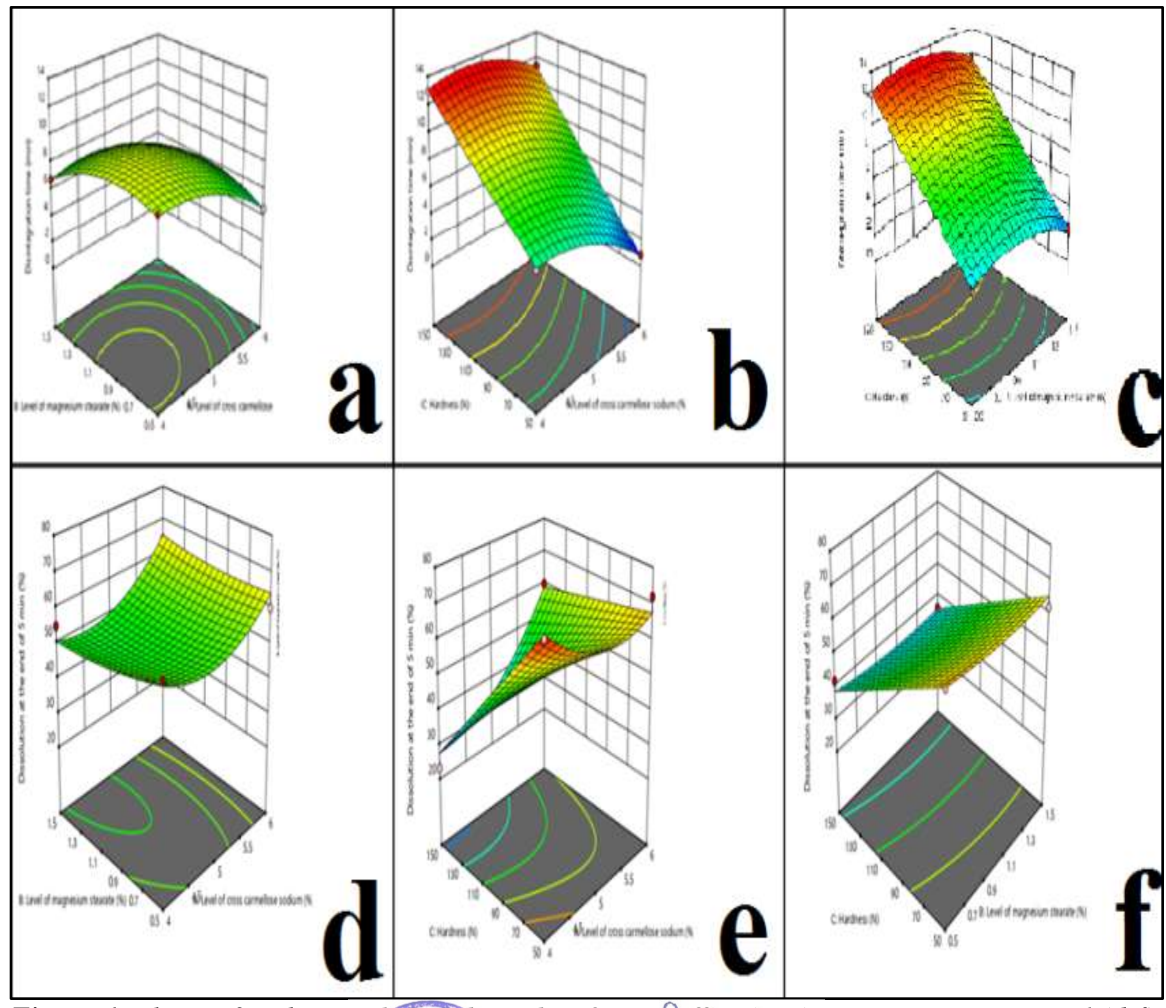

Figure 6: *Plots in 3D showing how independent factors affect (a–c): Disintegration time Y1 (d-f): drug release percentage Y2 (end of 2 minutes).*

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Design area: To find the factors that would have the biggest impact, a Box Behnken factorial design was performed. Based on the desirability and overlay plot, where the design space is shown by the yellow portion of the plot, the Design Expert software (V. 11.0.3.0) assessed the proposed concentrations of the independent variables (Fig. 7).

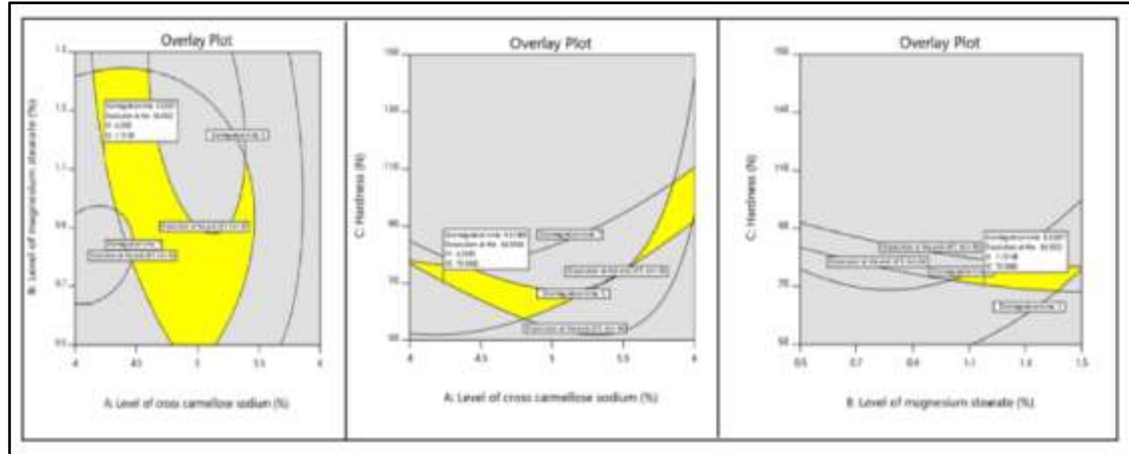

Figure 7: *Overlay plot of design space.*

The yellow region represents the simultaneous effects of independent factors on dependent variables (Fig. 7). According to the objectives shown in this multi-criteria decision technique employing QbD [22-25], the replies are within those targets.

Updated CQA risks include: The risk reduction and control plan are made up of several quality assurance methods based on knowledge about the product and the manufacturing process. All parameter values inside the design space's yellow zone suggest that they largely satisfy the QTPP. As a result, the initial risk was reduced from high to low in the revised risk assessment (Table 12).

Table 12: *Updated risk assessment for CQAs.*

Drug Product CQAs	Level of CCS	Level of Magnesium Stearate	Hardness
Disintegration time	Low*	Low	Low*
Dissolution	Low	Low*	Low*

To guarantee that a product would be regularly produced at the specified quality, a control strategy was created. The controls included a product specification process control and monitoring program and were based on an understanding of the product, formulation, and process. Variability-causing factors that affect product quality were found, correctly comprehended, and then managed. The final control method was used and is depicted in Table 13.

Table 13: *Control strategy for PT FDT.*

Measures	Range Studied	Set Point	Proposed Operating Range	Purpose of Control
Level of CCS	4-6%	5%	4.23-5.45%	To fulfill the criteria of IR tablet and drug release comparable to RLD
Level of magnesium stearate	0.5-1.5%	1%	0.50-1.42%	To fulfill the criteria of IR tablet and drug release comparable to RLD
Hardness	50-150N	100N	55.80-89.23N	To fulfill the criteria of IR tablet and drug release comparable to RLD

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Stability Studies: To investigate the effects of storage conditions on the dissolution profile, the optimized batch was placed in stable storage for duration of three months. We compared drug release characteristics to the initial release. The stability investigation revealed that the release profile of the formulation did not significantly alter, indicating that the therapeutic product had not degraded (Fig. 8).

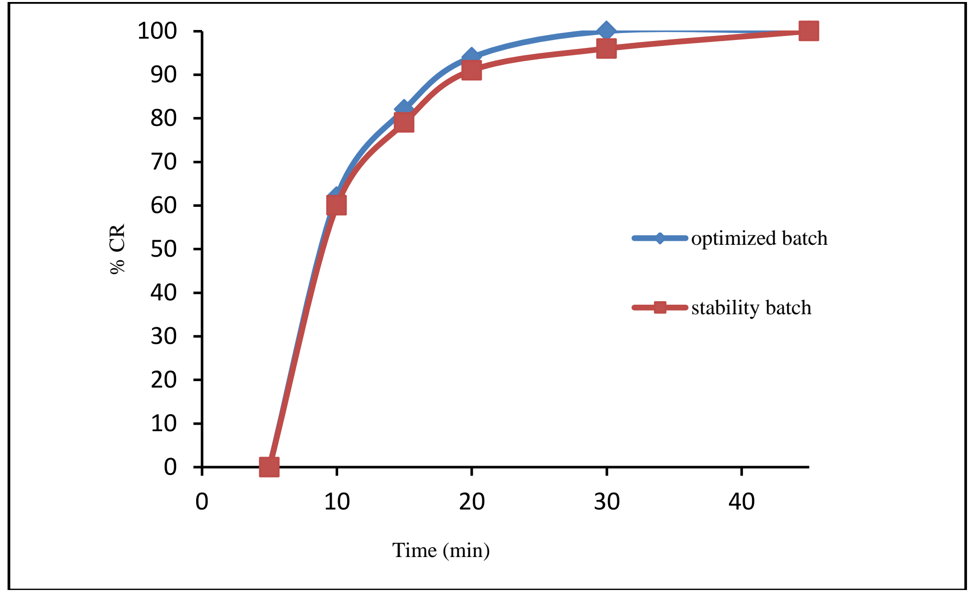

Figure 8: *% cumulative release of PT from optimized and stable batch.*

CONCLUSION

The creation of the PT-FDT formulation and in-vitro testing are the subjects of the current work. The development of a fast-dissolving tablet was accomplished thanks to the use of several core tablet compositions. Drug-excipient compatibility studies and the PT employed in the study both demonstrated compliance and no physical change. The batches' % medication release was calculated, and the results were compared to the intended target profile. The QbD technique was used to optimize the formulation for each parameter. For pharma products, important quality characteristics, QTPP design, identification of CQA, risk evaluation of CMA of PT formulation variable, and CPP were completed. For pharma product CQA, CMA, formulation variable, and CPP, updated risk assessments were created, and the risk was lowered from high to low. Comparison with commercial formulation was discovered to be within predetermined limits. A stability analysis was conducted, and the results revealed that the release profiles of the stability batch were equivalent to those of the first batch and that the pace and extent of the dissolution remained unchanged after the stability period.

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A Comparative Study on Antidiabetic Activity of *Gymnema Sylvestre*, Saxagliptin, Insulin and Alloherbal Combination in Alloxan Induced Diabetic Rats

PRATIK V. RANAWARE *, VITTHAL J. CHAWARE, ASHISH T. THORAT, VIVEKKUMAR K. REDASANI. *Department of Pharmacology, YSPM's Yashoda Technical Campus Faculty of pharmacy, Wadhe, Satara, India.*

ABSTRACT

Aim of the study **:** In this study, we evaluated and compared the effect of *Gymnema sylvestre*, Saxagliptin, Insulin and Alloherbal combination (*Gymnema sylvestre* & Saxagliptin) on hyperglycemia, plasma lipid profile, liver enzymes in Alloxan induced diabetes mellitus in rats.

Method**:** The antidiabetic activity (along with other parameters) of *Gymnema sylvestre* (100mg/kg), Saxagliptin (25mg/kg), Insulin (Human Actrapid; 10U/kg), Alloherbal combination (Gymnema sylvestre; 100mg/kg & Saxagliptin; 25mg/kg) was investigated in alloxan induced diabetes in rats. These drugs were administered once a day, for 14 days and blood glucose levels were measured on 0, 7 and 14th day. At the end of treatment various biochemical estimations & histopathological examination of pancreas were also carried out.

Result: The statistical data indicated, 14 Days oral administration all drugs included in study showed significant ($P<0.05$) decreased in blood glucose, total cholesterol, triglycerides, LDL; SGPT and SGOT level , along with significant increase in HDL; But not better than Alloherbal combination.

Conclusion : Present research findings provide experimental evidence that the combination of allopathic hypoglycemic drug; Saxagliptin with hypoglycemic herbal drug; *Gymnema sylvestre* provides effective and rapid glycemic control on diabetes mellitus and it could be considered for further evaluation in clinical studies and drug development.

Key words: *Gymnema sylvestre*, Saxagliptin, Insulin, Alloxan, Alloherbal combination, Diabetes

1. INTRODUCTION

Diabetes mellitus (DM) It is a metabolic disorder characterized by hyperglycaemia, (fasting plasma glucose ≥ 126 mg/dL and/or > 200 mg/dL 2 hours after 75 g oral glucose), glycosuria, hyperlipidaemia, negative nitrogen balance and sometimes ketonaemia. A widespread pathological change is thickening of capillary basement

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membrane, increase in vessel wall matrix and cellular proliferation resulting in vascular complications like lumen narrowing, early atherosclerosis, sclerosis of glomerular capillaries, retinopathy, neuropathy and peripheral vascular insufficiency.[1]

Gymnema sylvestre[2] is a perennial woody vine native to Asia (including the Arabian Peninsula), Africa and Australia. It has been used in Ayurvedic medicine. Common names include gymnema,[3] Australian cowplant, and Periploca of the woods, and the Hindi term *gurmar*, which means "sugar destroyer".[4][5][6] It has significant antidiabetic as well as hypolipidemic activity so that it can be used as an adjuvant along with allopathic treatment of medicine to treat diabetes as well as to delay the late complications of diabetes.[7]

Saxagliptin a Dipeptidyl Peptidase-4 (DPP-4) inhibitor are the newer class of compounds that was approved in 2006 for the treatment of T2DM. Their primary mechanism of action is through inhibition of degradation of incretins, such as glucagon like peptide-1 (GLP-1) and Gastric Inhibitory Polypeptide (GIP)[8]

Human Actrapid is a fast-acting insulin. Onset of action is within ½ hour, reaches a maximum effect within 1.5–3.5 hours and the entire duration of action is approximately 7–8 hours.The blood glucose lowering effect of insulin is due to the facilitated uptake of glucose following binding of insulin to receptors on muscle and fat cells and to the simultaneous inhibition of glucose output from the liver. [9]

This work reviews and comparatively analyzes the herbal, allopathic and biologic treatments to cure the problems in health care. It suggests the adoption of the concept of integrative medication and health care that connects mainstream allopathic medical treatment, herbal therapies and biologics, which will select the best, scientifically validated therapies out of the systems.

2. MATERIALS AND METHODS:

2.1 Drugs and Chemicals:

Alloxan monohydrate obtained from Dolphin pharmacy instruments Pvt. Ltd. Mumbai. *Gymnema sylvestre* obtained from Inlife pharma Pvt. Ltd. Saxagliptin obtained from CTX Lifesciences Pvt. Ltd. Gujrat. Human Actrapid Insulin 40IU/ml obtained from novo nordisk®

2.2 Animals & Housing Condition :

Albino Wistar Rats of (180-200gm) were selected for experimental study. The animals were kept and maintained under laboratory conditions of temperature 22 ± 2°C, relative humidity 50± 15% and 12 hrs. light/dark cycle. They were allowed free access to food (standard pellets) and water *ad libitum.* Experimental protocols and procedures used in this study were approved by the Institutional Animal Ethics Committee of YSPM's, YTC, Faculty of Pharmacy, NH4 Wadhe, Satara, Maharashtra, India.

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2.3 Induction of Diabetes:

Albino Wistar Rats were made diabetic by a single intraperitoneal injection of Alloxan monohydrate (150 mg/kg/day). Alloxan monohydrate solution of 150mg/kg/day prepared in 0.9% NaCl solution and was administered within 5 minutes at a dose of 150-mg/kg/day intraperitoneally. All the animals except control group were i.p. administered with Alloxan at a dose of 150mg/kg once a day for 2 days. After 72 hours of Alloxan administration, rats with moderate diabetes having glycosuria and hyperglycemia (i.e. with a blood glucose of 250- 350mg/dl) were taken for the experiment. **[10]**

2.4 Blood Glucose level & Body Weight Determination:

Blood samples were drawn from tail tip of rats. Fasting blood glucose estimation were done on 0th, 7th, & 14th day of the study. Blood glucose estimation was done by ACCU-CHECK Active Glucometer using glucose test strips. For body weight determination, all experimental animals were weighted on 0th, 7th, & 14th day of the study. The body weights were recorded at recording time in the morning mentioned by Al-Attar and Zari **[11]**. Furthermore, for any signs of abnormalities throughout the duration of investigation, the rats were continuously observed.

2.5 Biochemical Estimation:

After Fourteen days, rats were fasted for 8 h. Rats were anesthetized using diethyl ether and samples of blood were obtained from retro-orbital plexus. These Blood samples were withdrawn for estimation of Blood glucose level, Lipid profile (Total cholesterol, Triglycerides, HDL, LDL, VLDL), Liver function test (Alkaline phosphatase, AST;SGOT, ALT;SGPT, Total Protein etc.).

2.6 Histopathological Examination:

After blood collection, all rats were sacrificed with high dose of anaesthesia and dissected; pancreatic tissues were isolated and fixed in 10% formalin. Fixed pancreatic tissues were dehydrated and embedded in paraffin. All tissues were sectioned at 4 µm. The routine process of staining was applied using hematoxylin and eosin stains **[12]**. The pancreatic sections were evaluated by light microscopy using Motic basic biological microscope BA210. Motic imaging software was used to evaluate the histological profile of pancreatic sections in all groups.

2.7 Experimental Design:

2.7.1 Acute Toxicity Study:

Acute toxicity study was carried out for Gymnema sylvestre by adapting fixed dose method of CPCSEA, OECD guidelines no. 423. Healthy Albino Wistar rats of either sex were randomly divided into 4 groups with 3 animals in each group. The animals were kept fasted overnight providing only water, after which the *Gymnema sylvestre* were administered orally with Starting dose is selected from one of four fixed levels 5, 50, 300, and 2000 mg/kg body weight by intra gastrictube. Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily there after, for a total of 14 days. Animals are observed for general neurological & behavioural or autonomic profile. **[13]**

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2.7.2 Hypoglycemic Evaluation:

For Hypoglycemic evaluation, Albino Wistar Rats were used and divided into five groups of six animals in each group. Animals were kept fasted overnight (18hrs) before treatment.

Group I - (Control) rats received vehicle that was Distilled water (10ml/kg p.o.).

Group II - (Test1) rats received *Gymnema sylvestre* (100mg/kg p.o.)

Group III - (Test2) rats received Saxagliptin (25mg/kg p.o.)

Group IV - (Test3) rats received Insulin (1U/100gm SC).

Group V - (Test4) rats received *Gymnema sylvestre* (100mg/kg p.o.) and Saxagliptin (25mg/kg p.o.) in combination.

Blood glucose was estimated on 0, 30, 60, 90, 120 min of the treatment using the ACCU-CHECK Active Glucometer.

2.7.3 Oral Glucose Tolerance Test:

For OGTT evaluation, Albino Wistar Rats were used and divided into five groups of sixanimals in each group. Animals were kept fasted overnight (18hrs.) before treatment.

Group I- (Control) rats received Glucose (2gm/kg p.o.)

Group II- (Test1) rats received *Gymnema sylvestre* leaves extract (100mg/kg p.o.)

Group III- (Test2) rats received Saxagliptin (25mg/kg p.o.)

Group IV- (Test3) rats received Insulin (1U/100gm SC).

Group V- (Test4) rats received *Gymnema sylvestre* leaves extract (100mg/kg p.o.) andSaxagliptin (25mg/kg p.o.) in combination.

Glucose (2gm/kg p.o.) was administered to all the rats after Half hour of administration of differentdrug treatments. Blood glucose was estimated at 0, 30, 60, 90 & 120 min after different drug treatment using the ACCU-CHECK Active Glucometer.

2.7.4 Antidiabetic study by different drug treatment :

After 72 hours of Alloxan (150mg/kg/day i.p.) administration, rats with moderate diabetes havingglycosuria and hyperglycemia (i.e. with a blood glucose of 250-350 mg/dl) were taken for the experiment. The Albino Wistar rats were divided into six groups of six rats in each group. All the animals were fasted overnight (18hrs.) before the treatment of test drug.

Group I- (Normal Control) rats received only vehicle that is Distilled water (10ml/kg/day)

Group II- (Toxic Control) rats received Alloxan Monohydrate (150mg/kg/day)

Group III- (Test 1) rats received *Gymnema sylvestre* leaves extract (100mg/kg p.o.)

Group IV- (Test 2) rats received Saxagliptin (25mg/kg/day p.o.)

Group V- (Test 3) rats received Insulin (1U/100gm/day SC).

Group VI- (Test 4) rats received *Gymnema sylvestre* leaves extract (100mg/kg/day p.o.) and Saxagliptin (25mg/kg/day p.o.) in combination.

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2.8 Statistical Analysis :

All values of results were presented as mean ± standard error of mean (SEM). The statistical analysis involving one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison posttest was used for statistical comparison between control and various treated groups. Statistical significance was accepted at the $p < 0.05$ values.

3. RESULTS

3.1 Hypoglycemic Effect of different drug treatment in Normal Rats:

The results from the study clearly indicated that the administration of *Gymnema sylvestre*, Saxagliptin, Insulin and *Gymnema sylvestre* with Saxagliptin (combination) reduced the blood glucose level significantly on 90 and 120min as compared with normal control group.

Table 1: Hypoglycemic Effect of different drug treatment in Normal Rats

Group no.	Treatment Groups (n=6)	Fasting Blood Glucose Level (mg/dl)				
		0 min	30min	60min	90min	120min
I	NormalControl	67±1.78	70±1.89	66±1.88	67±3.10	66±2.29
II	Test group 1 (*Gymnema sylvestre*)	73±1.73	71±2.11	69±2.36	65±1.92	60±3.15
III	Test group 2 (Saxagliptin)	81±2.43	71±2.39	63±2.01	57±2.36	55±1.87
IV	Test group 3 (Insulin)	90±2.46	68±1.89	53±2.79	56±2.30	48±3.48
V	Test group 4 (G.S + Saxagliptin)	71±2.03	65±2.76	63±3.07	60±2.88	58±2.15

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnett's multiple comparison test. ($^*p < 0.05$).

3.2 Effect of different drug treatment on the Oral Glucose Tolerance Test in Normal Rats:

The results from the study, clearly indicated that the administration of *Gymnema sylvestre*, Saxagliptin, Insulin and *Gymnema sylvestre* with Saxagliptin (combination) reduced the blood glucose level (hyperglycemia due to glucose load (2g/kg p.o.) significantly after 120 min of administration, as compared with control group.

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Table 2: Effect of different drug treatment on the Oral Glucose Tolerance Test in NormalRats

Group no.	Treatment Groups (n=6)	Fasting Blood Glucose Level (mg/dl)				
		0 min	30min	60min	90min	120min
I	Control (Glucose)	80±1.54	78±2.55	130±1.67	110±1.89	94±1.56
II	Test group 1 (*Gymnema sylvestre*)	85±1.25	82±1.99	120±3.89	109±2.56	90±1.59
III	Test group 2 (Saxagliptin)	88±1.55	83±2.45	110±1.98	95±1.22	85±3.68
IV	Test group 3 (Insulin)	87±1.98	70±3.56	82±1.36	62±2.66	41±3.02
V	Test group 4 (G.S + Saxagliptin)	90±1.5	83±3.01	111±2.65	95±1.98	82±1.75

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnett's multiple comparison test. ($^*p < 0.05$).

3.3 Effect of different drug treatment on Body Weight of Diabetic Rats:

At the end of study after 14 days, body weight was significantly decreased in toxic control groupas compared with normal control group & significantly increased in *Gymnema sylvestre*, Saxagliptin, Insulin and *Gymnema sylvestre* with Saxagliptin (combination) treated group as compared with toxic control group. But Insulin treated group shows marked rise in body weight than the Normal control group.

Table 3: Effect of different drug treatment on Body Weight of Diabetic Rats

Group no.	Treatment Groups (n=6)	Body Weight of Animals (gm)		
		0th day	7th day	14th day
I	Normal control	210±1.33	215±1.89	224±2.01
II	Toxic control	216±1.65	196±2.96	180±3.69
III	Test group 1	228±2.63	232±3.06	238±1.32
IV	Test group 2	237±3.01	242±1.65	249±2.36
V	Test group 3	241±2.65	252±3.11	266±1.158
VI	Test group 4	248±1.65	253±2.36	259±3.05

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnett's multiple comparison test. ($^*p < 0.05$).

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3.4 Effect of different drug treatment on Fasting Blood Glucose Level in Diabetic Rats

A marked rise in fasting blood glucose level was observed in toxic control group as compared with normal control group. *Gymnema sylvestre* (100mg/kg) And Saxagliptin (25mg/kg) treated group which produced a significant reduction in blood glucose level as compared with toxic control group; But not better than their combination. Where the Insulin treated group shows Goodcontrol of Hyperglycemia until 7th day then it shows mild Hypoglycemia on 14th day.

Table 4: Effect of different drug treatment on Fasting Blood Glucose Level in Diabetic Rats

Group no.	Treatment Groups (n=6)	Fasting Blood Glucose Level (mg/dl)		
		0th day	7th day	14th day
I	Normal Control	80±1.89	80±1.045	82±2.07
II	Toxic control	321±2.89	326±1.22	330±1.65
III	Test group 1 (*Gymnema sylvestre*)	285±1.59	221±1.03	157±2.67
IV	Test group 2 (Saxagliptin)	299±2.86	191±1.09	144±1.75
V	Test group 3(Insulin)	325±1.65	195±2.69	65±1.44
VI	Test group 4 (G.S + Saxagliptin)	316±1.07	165±1.76	115±2.66

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnett's multiple comparison test. ($^{*}p < 0.05$).

3.5 Effect of different drug treatment on Biochemical Parameters in Diabetic Rats:

Serum Lipid Profile:

After 14 days of treatment period it was observed that increased level of Total Cholesterol, TG, LDL, VLDL, & decreased HDL level in toxic control group as compared with normal control group. Animals treated with *Gymnema sylvestre* (100mg/kg) And Saxagliptin (25mg/kg) showedsignificant reductions in Total Cholesterol, LDL, VLDL, TG & significant increased level in HDLas compared with toxic control group; But not better than their combination (i.e. G.S + Saxagliptin). Where Test group 3 (i.e. Insulin) showed reduction in values than normal control values.

Table 5 : Effect of different drug treatment on Biochemical Parameters in Diabetic Rats

Group no.	Treatment Groups (n=6)	Total Cholesterol (mg/dl)	Tri-Glycerides (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)
I	NormalControl	78.4±1.54	122±2.03	11.9±1.98	39±1.09	24±1.45
II	Toxic control	102±2.13	480±1.98	38±3.54	35±4.03	96±1.84
III	Test group 1 (*Gymnema sylvestre*)	87±3.05	153±2.03	18.3±1.04	37±4.65	30.7±2.51
IV	Test group 2 (Saxagliptin)	100±2.65	140±3.98	24.8±1.75	34±4.03	28±1.33
V	Test group 3 (Insulin)	66±2.68	201±1.66	21±3.78	30±4.65	40.2±1.21
VI	Test group 4 (G.S + Saxagliptin)	83±1.03	120±2.78	19±1.98	39±3.98	24±4.01

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followedby Dunnett's multiple comparison test. ($^{*}p < 0.05$).

3.6 Effect of different drug treatment on Liver Function Test in Diabetic Rats :

After 14 days of treatment period it was observed that increased level of Bilirubin, SGPT, SGOT,TP & ALKP in toxic control group as compared with normal control group. Animals treated with*Gymnema sylvestre* (100mg/kg) And Saxagliptin (25mg/kg) showed significant reductions in AST; SGOT, ALT; SGPT & ALP as compared with Toxic control group; But not not better thantheir combination (i.e. G.S + Saxagliptin). Where Test group 3 (i.e. Insulin) shows reduction in values than normal Control values.

Table 6 : Effect of different drug treatment on Liver Function Test

Group no.	Treatment Groups (n=6)	Bilirubin mg/dl	SGPT U/L	SGOT U/L	TP mg/dl	ALKP U/L
I	NormalControl	0.74±1.33	43±1.6	36±3.06	5.8±2.09	126±1.48
II	Toxic control	1.12±2.35	59±1.59	42±3.65	7.5±4.89	190±2.89
III	Test group 1 (*Gymnema sylvestre*)	0.83±1.78	46±2.13	36±3.60	6.5±4.21	110±2.44
IV	Test group 2 (Saxagliptin)	0.93±2.54	38±3.45	31±1.09	6.9±4.01	102±1.98
V	Test group 3 (Insulin)	0.6±1.08	34±2.40	29±1.88	4.9±4.02	97±3.48 445

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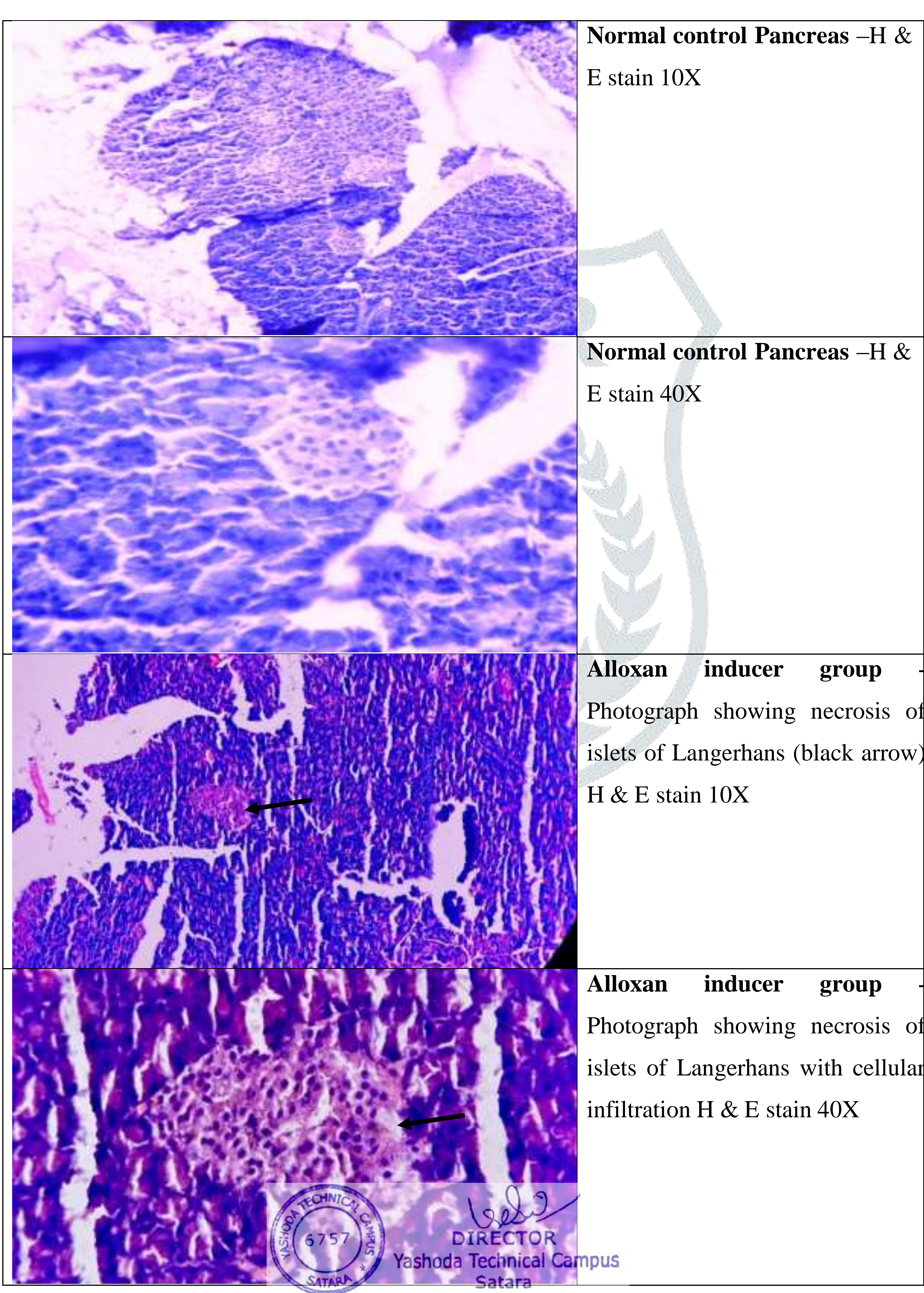
VI	Test group 4(G.S + Saxagliptin)	0.79±1.03	39±2.03	30±2.78	6.9±3.33	118±4.11

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnett's multiple comparison test. ($^{*}p < 0.05$).

Pancreas Histopathology



Normal control Pancreas –H & E stain 10X

Normal control Pancreas –H & E stain 40X

Alloxan inducer group - Photograph showing necrosis of islets of Langerhans (black arrow) H & E stain 10X

Alloxan inducer group - Photograph showing necrosis of islets of Langerhans with cellular infiltration H & E stain 40X

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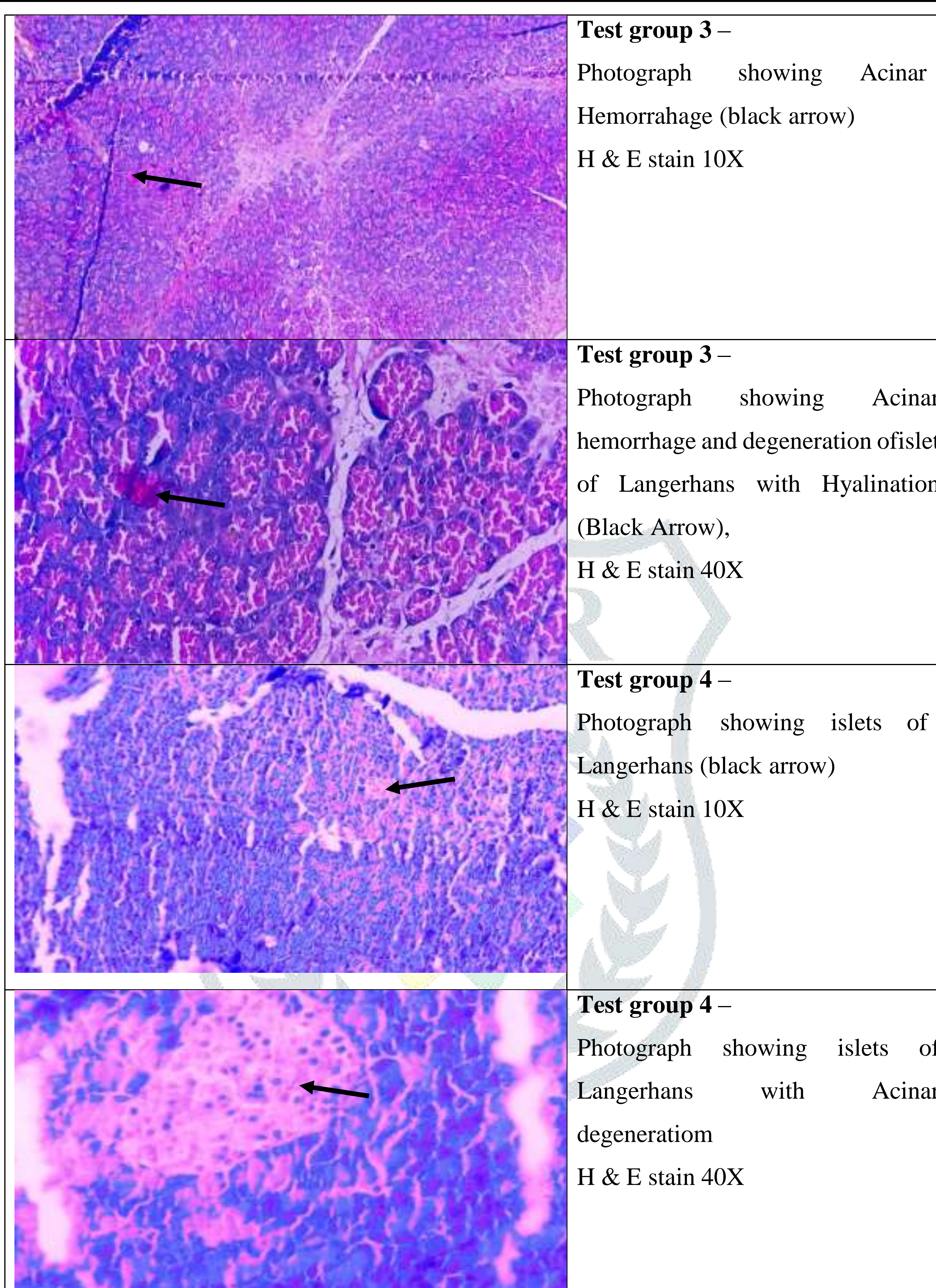
	Test group 1 – Photograph showing Acinar and degeneration of islet of Langerhans (Black Arrow), H & E stain 10X
	Test group 1 – Photograph showing Acinar and degeneration of islet of Langerhans (Black Arrow), H & E stain 40X
	Test group 2 – Photograph showing necrosis of islets of Langerhans (black arrow),H & E stain 10X
	Test group 2 – Photograph showing necrosis of islets of Langerhans with cellular infiltration (black arrow) H & E stain 40X



Test group 3 –
Photograph showing Acinar Hemorrahage (black arrow)
H & E stain 10X

Test group 3 –
Photograph showing Acinar hemorrhage and degeneration ofislet of Langerhans with Hyalination (Black Arrow),
H & E stain 40X

Test group 4 –
Photograph showing islets of Langerhans (black arrow)
H & E stain 10X

Test group 4 –
Photograph showing islets of Langerhans with Acinar degeneratiom
H & E stain 40X

4. DISCUSSION :

4.1 Acute oral toxicity, Hypoglycemic study, OGTT Study & Body Weight Determination:

Globally, the rapid increase the incidence of DM poses a demand for the quest of novel therapeuticdrugs necessitates addition of alternative medicine. As a result number of studies has been conductedto assess the utility of herbal and allopathic medicine in DM. The present study was undertaken to evaluate the Antidiabetic activity of *Gymnema sylvestre*, Saxagliptin, Insulin and Alloherbal combination against Alloxan Induced Diabetic Albino Wistar Rats.

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The Acute oral toxicity was performed according to OECD guideline 423. In this study we observed that the *Gymnema sylvestre* was safe to use in animals. There was no change in neurological, behavioural or autonomic, no lethality or toxic reactions were found with the selecteddoses (5, 50, 300 and 2000mg/kg/day p.o.) until the end of study period. Therefore 100 mg/kg was selected for all in vivo experiments as minimal dose.

The results of Hypoglycemic study have shown that the administration of *Gymnema sylvestre*, Saxagliptin, Insulin and *Gymnema sylvestre* with Saxagliptin (combination) reduced the blood glucose level significantly on 120min as compared with normal control group.(Table 3).

OGTT for nondiabetic rats were performed according to the standard method (Du Vigneaud and Karr, 1925).[14] The Oral glucose tolerance test in nondiabeticc rats, blood glucose level was significantly greater in the glucose loaded control group. The results from the study, clearly indicated that the administration of *Gymnema sylvestre*, Saxagliptin, Insulin and *Gymnema sylvestre* with Saxagliptin (combination) reduced the blood glucose level (hyperglycemia due to glucose load (2gm/kg p.o.) after120 min of administration, as compared with control group. (Table 4).

Induction of diabetes by Alloxan leads to loss of body weight due to increased muscle wasting and loss of tissue proteins as well as due to destruction of pancreatic cells; insufficient insulin prevents the body from getting glucose from the blood into the body's cells to use as energy & when this occurs, the body starts burning fat and muscle for energy, causing a reduction in overall body weight.)[15] whereas body weight of animals significantly increased in *Gymnema sylvestre*, Saxagliptin and their combination treated group as compared with toxic control group. But treatmentof Insulin shows marked rise in body weight.).[16] (Table 5).

4.2 Alloxan-Induced Rodent Model of Diabetes & Antidiabetic effect of different drug treatment:

Alloxan has two distinct pathological effects: Alloxan is a toxic glucose analogue it selectively inhibits glucose-induced insulin secretion through specific inhibition of glucokinase, the glucose sensor of the beta cell, and it causes a state of insulin-dependent diabetes through its ability to induceROS formation leading to demolition of pancreas β-cells & selective necrosis leading to hypoinsulinemia and hyperglycemia.

The results of the antidiabetic study reduced blood glucose level Significantly on 7th & 14th days when animals treated with *Gymnema sylvestre*, Saxagliptin, Insulin and *Gymnema sylvestre* with Saxagliptin (combination) as compared to toxic control groups (Table 6)

There are some possible mechanisms by which the *Gymnema sylvestre* leaves and especially Gymnemic acids from G. sylvestre exert its hypoglycemic effects are: 1) it increases secretion of insulin, 2) it promotes regeneration of islet cells, 3) it increases utilization of glucose: it is shown toincrease the activities of enzymes responsible for utilization of glucose by insulin dependant pathways, an increase in phosphorylase activity, decrease in gluconeogenic enzymes and sorbitol dehydrogenase, and 4) it causes inhibition of glucose absorption from intestine).[17]

Saxagliptin is part of a class of diabetes medications called dipeptidyl peptidase-4 (DPP-4) inhibitors. DPP-4 is an enzyme that breaks down incretin hormones. As a DPP-4 inhibitor, saxagliptin slows down the breakdown of incretin hormones, increasing the level of these hormonesin the body. It is this increase in incretin hormones that

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is responsible for the beneficial actions of saxagliptin, including increasing insulin production in response to meals and decreasing the rateof gluconeogenesis in the liver).[18] Dipeptidyl peptidase-4's role in blood glucose regulation is thought to be through degradation of GIP and the degradation of GLP-1).[19][20]

The blood glucose lowering effect of insulin is due to the facilitated uptake of glucose following binding of insulin to receptors on muscle and fat cells and to the simultaneous inhibition of glucose output from the liver).[21]

4.3 Biochemical Parameters Analysis:

In Alloxan induced diabetes mellitus showed improvement in biochemical parameters after the treatment of Drugs.

In the result of **lipid profile**, marked decrease in total cholesterol, LDL, VLDL and triglycerides was observed, while increase in HDL cholesterol which reduces the risk of atherosclerosis has been observed in *Gymnema sylvestre*, Saxagliptin, Insulin and *Gymnema sylvestre* with Saxagliptin (combination) treated diabetic rats, which suggest that HDL is inversely related to the total body cholesterol as compared with toxic control group (Table 7).

These results could thus reflect the ability of *Gymnema sylvestre*, Saxagliptin improve the tissue sensitivity to insulin. Thus reducing the hormone sensitive lipase activity and increasing the lipoprotein lipase activity, resulting in a decrease of lipolysis these leading to hypolipidemic activity.

In the present study, rats treated with G. sylvestre post Alloxan-diabetic induction showed a significant decrease in triglyceride, cholesterol and LDL and showed a significant increase in HDLas compared to that of untreated diabetic rats. Decreasing levels of triglyceride, cholesterol and LDLand increasing level of HDL might be due to an increase in insulin which caused an increased activity of lipoprotein lipase (Facilitated chylomicron transport through cell membranes) and a decreased activity of hormone-sensitive lipase (converted neutral fats into free fatty acids). This result was in agreement with Daisy et al. (2009) [22] and Aralelimath and Bhisea (2012) [23] who reported that increasing insulin secretion after administration of G. sylvestre extract led to a decreaseof cholesterogenesis and fatty acid synthesis.

Our study results elucidated that, saxagliptin improve lipid status in rats, via significant reduction of Total Cholesterol, LDL and Triglycerides. In line with these results are also the results of other research groups. Possible explanation for beneficial lipid effects of DPP4 inhibitors may be connected to its stimulating effect on the activated proteine-kinase pathway, which leads to increasein glucose and lipid catabolism. [24] On the other hand, no improvement in HDL parameters was achieved in our study, which is in correlation with the findings of Saad et al. [25]

In our study the result of Insulin treatment is accordance with Ibrahim Aslan et.al. (2013) [26] whichshows Total cholesterol (TC), triglyceride (TG) and very low-density lipoprotein cholesterol (VLDL-C) levels were significantly decreased while HDL-C levels were significantly increased after insulin treatment.

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In **liver function test**, animals treated with *Gymnema sylvestre*, Saxagliptin, Insulin and *Gymnema sylvestre* with Saxagliptin (combination) treated group showed significant reductions in Bilirubin, ALT; SGPT, AST; SGOT, Total Protein & ALKP as compared with toxic control group (Table 8). This demonstrated the hepatoprotective activity could be related to reduced blood glucose level due to different treatment groups.

Evidence from studies about heme oxygenase (HO) system[27][28] might also support the increasedrisk of bilirubin with T2D. Increased activity of HO could elevate the heme catabolic products suchas carbo monoxide, iron, and bilirubin. [27] HO-1 has been reported as a strong positive predictor ofmetabolic inflammation among obese insulin-resistance individuals and animals.[28][29] The higher bilirubin levels might be a biomarker of oxidative stress and inflammation in diabetes

Glucose level might be decreased in treated diabetic rats as a result of decreasing gluconeogenesis that was indicated by low levels of ALT;SGPT and AST;SGOT in treated diabetic rats compared tountreated diabetic rats(Toxic Control Group). This result was in agreement with Shanmugasundaramet al. (1983)[30] who reported that administration of dried leaf powder of G. sylvestre decreased glucose levels as it controlled gluconeogenic enzymes (ALT and AST) and increased glycogen levels in liver, kidney and muscle.

Total proteins were found to be significantly increased in diabetics as compared to Normal controls.Competition between serum albumin and hemoglobin could be a factor for the negative correlationbetween them, besides preventing other proteins from glycation and altering the diabetic complications. Similar findings have been reported by other studies.[31] [32] [33] [34] Increase in total proteins may be due to the elevation of acute phase proteins, globulins, fibrinogen and compounded by decrease in the fractional synthetic rate of albumin due to insulin resistance/deficiency (F A Nazki 2017)

4.4 Histopathological Examination:

In histopathological study (Table 19), the fine section of Normal Control diabetic rat's pancreas onmicroscopic examination using H & E stain, 10X & 40X showed the presence of islets of Langerhans, blood vessels, connective tissues, inter and arrangement of islets of Langerhans was normal with tightly arranged cells and even distribution throughout the lob-necrosis.

Also, Pancrease Exocrine portion predominantly and composed of lobules, each of which is surrounded by connective tissue septa through which run blood vessels, nerves, lymphatics, and interlobular ducts. Adequate islets of beta and alfa cells was seen. No evidence of stromal Infiltrationwas seen.

In toxic control group i.e Alloxan inducer group Histopathological report shows that necrosis of islets of Langerhans was shown with cellular infiltration. It also Shows Exocrine portion predominantly and composed of lobules formed by acinar structure, each of which is surrounded byconnective tissue septa through which run blood vessels, nerves, lymphatics, and interlobular ducts.

There is No evidence of islets of beta and alfa cells are seen, which complete destruction of alpha and beta cells.

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In Saxagliptin treated diabetic rats, Photograph showing necrosis of islets of Langerhans with cellular infiltration (The migration of cells from their sources of origin), Where In Insulin treated rats showing Acinar hemorrhage

and degeneration of islet of Langerhans with Hyalinization (process of conversion of stromal connective tissue into a homogeneous, acellular translucent material that could provide insights into the prognosis of pathological lesions.) was seen. So it can be concluded that Insulin treatment does not provide protection to the pancreatic cells.

In *Gymnema sylvestre* and *Gymnema sylvestre* with Saxagliptin treated groups it was observed thatalthough the gap between the islets was more than lesser number of islets as compared to Normal control group, it was significantly much better than the toxic control group. By showing Acinar degeneration it is concluded that, the dose of *Gymnema sylvestre* had slight protection to the cells. Because Necrosis formed after Acinar degeneration which was shown in Toxic control group. Thus the histopathological examination revealed good protective property of this herbal drug alone and with combination.

5. CONCLUSION :

In conclusion, it can be stated that the use of Combination of *Gymnema sylvestre* and Saxagliptin produces more beneficial effect than using alone. Gymnemic acids from G. sylvestre exert its hypoglycemic effectsby increasing secretion of insulin, increasing utilization of glucose or inhibiting glucose uptake from intestine; Whereas Saxagliptin exerts its hypoglycemic effect by this increase in incretin hormonesand increasing insulin production in response to meals and decreasing the rate of gluconeogenesis inthe liver.

Use of *Gymnema sylvestre* and Saxagliptin in combination shown beneficial effects in reducing theelevated blood glucose level as well as gained body weight, hypoglycemic potential, significant oral glucose tolerance & normalization in altered biochemical parameters of Alloxan induced diabetic rats.

From the present results it can be concluded that using herbs with allopathic drugs may produce the synergistic effect. Hence, For further use of these combination clinical studies are needed.

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Life Cycle Management of Analytical RP-HPLC Method Development for Assay of Rizatriptan in Immediate Release Dosage Form

Mr. Avinash M. Bhagwat[1]*
Dr. Rahul V. Mayee[2]
Dr. Ajit B. Ekal[3]

[1]Assistant Professor, YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara
[2]Head MW & RA, BioRadius Therapeutic Research Pvt. Ltd. Pune
[3]Managing Director, Instavision laboratory and Services, Satara
Dept. of Pharmaceutical Chemistry
YSPM's Yashoda Technical Campus, Wadhe, Satara 415 001

Abstract:
In this research work of life cycle management of analytical RP-HPLC method development for assay of Rizatriptan in immediate release dosage form, the RP-HPLC assay method was developed and validated for Rizatriptan. Stress study is also carried out. The chromatographic conditions will be as, Symmetry C-18, 150 mm x 4.6 mm, 5 µm column at 30°C temperature by using a mobile phase [Mixture of 100 mL Acetonitrile, and 900 mL Buffer solution] at a flow rate of 1.8mL/min, and UV detection at 225 nm and run time is 15 min. This life cycle management stability indicating RP-HPLC analytical method is economical, specific, accurate, precise and robust for assay of Rizatriptan in immediate release dosage form.
***Key words*: Rizatriptan, Life Cycle Management, RP-HPLC Method Development, Validation, Immediate Release Dosage Form**

Materials and Methods:
Introduction:
Rizatriptan benzoate is N,N-dimethyl-2-[5-(1H-1,2,4-triazol-1-ylmethyl)-1H-indol-3-yl]ethanamine. It is an anti migraine drug, which selectively activates 5-HT1B/1D receptors. Physical properties are white to off white crystalline powder, soluble in water, melting point 178–180_, and stable under ordinary condition. The some published methods of analysis for determination and separation of Rizatriptan in their formulation were not evaluated for specificity and degradation study. Therefore, method having specificity for degradation products and formulation excipients is considered as a prime requirement. Degraded samples, prepared by systematic forced degradation study, were used for method development trials to optimize the method as a stability indicating method for determination of Rizatriptan. This life cycle management stability indicating RP-HPLC analytical method is economical, specific, accurate, precise and robust for assay of Rizatriptan in immediate release dosage form.

Structure of Rizatriptan benzoate
Experimental:
Materials:
1) Risatriptan (Rizatriptan Benzoate): - Working standard and its claimed purity was 98.20%.
2) Risatriptan (Rizatriptan Benzoate) Tablet (label claim 5 and 10 mg) and placebo, which was prepared and supplied by Instavision lab.

- Reagents and Chemicals:
  1) Acetonitrile: -HPLC grade, Rankem, India.
  2) Methanol: - HPLC grade, Rankem, India.
  3) Milli-Q water: - It was purified by Millipore Corporation's system.
  4) Acetic acid: - Reagent Grade, Merck, India.
  5) Hydrochloric acid: - Reagent Grade, Merck, India.
  6) Sodium hydroxide: - Reagent Grade, Merck, India.
  7) Hydrogen Peroxide (30%):- Reagent Grade, Merck, India.
  8) Sodium Perchlorate :- Reagent Grade, Merck, India.
  9) Triethylamine :- Reagent Grade, Merck, India.

Instruments, Apparatus and equipment:

1) High Performance Liquid chromatography system (HPLC): Agilent Liquid Chromatography with PDA detector
2) Chromatographic software:– E Z Crome Elite
3) A double beam UV-visible spectrophotometer having two matched cells with 1cm light path: - UV- 2450, Shimadzu, Japan.

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4) Analytical Balance: - AD 265S, Mettler Toledo, Sweetzerland.
5) pH Meter: - Labindia, India.
6) Sonicator: - 5510, Branson Ultrasonics Corporation, Danbury, CT, USA.
7) Hot air oven: - Labline, India.
8) Photo stability chamber: - SVI equipments, Germany

➢ Chromatographic system:

Degradation studies were carried out on a system consisted of 1200 series HPLC (Agilent Technologies) comprising of an on-line degasser (G1322A), binary pump (G1312A), auto injector (G1367C), column oven (G1310B), DAD detector (G1315C) and E Z Crome Elite (software).

The published methods of analysis for determination and separation of Rizatriptan in their formulation were not evaluated for specificity and degradation study. Therefore, method having specificity for degradation products and formulation excipients is considered as a prime requirement. Degraded samples, prepared by systematic forced degradation study, were used for method development trials to optimize the method as a stability indicating method for determination of Rizatriptan.

➢ Selection of Buffer in Mobile Phase: -

Sodium perchlorate and 1ml Triethylamine in 1000ml water and adjusted pH 2.00, 3.00, 4.00 and 5.00 with 10% Acetic acid was used to optimize the peak shape and to proper separation of impurities peaks from main drugs peaks. The ratio of (Buffer: Acetonitrile) was selected on the basis of resolution between the major degradation peaks and main peaks, and it was finalized as (90:10) v/v after analyzing all the degraded samples and evaluating the peak purity, resolution, specificity and stability indicating nature of the method.

➢ Selection of Mobile Phase: -

Acetonitrile was used to optimize the retention time of late eluting impurities and Methanol to proper separation of impurities peaks from main drugs peaks. The ratio of (Buffer: Acetonitrile) was selected on the basis of resolution between the major degradation peaks and main peaks, and it was finalized as (90:10) v/v after analyzing all the degraded samples and evaluating the peak purity, resolution, specificity and stability indicating nature of the method.

➢ Selection of Column:-

For HPLC, various columns are available, but as the main aim of the method to resolve the compound in the presence of degradation products and impurities, a reversed phase C_{18} column was preferred over other columns to separate all polar impurities as Symmetry C-18, 150 mm x 4.6 mm, 5 µm column was chosen to give good peak shape, good lifetime and high resolution on compared to other C_{18} columns.

➢ Selection of Diluent / Solvent for extraction:-

Different solvents were tried including single solvent and combination of solvents like Acetonitrile and methanol in different concentrations, But Risatriptan (Rizatriptan Benzoate) tablet gets dissolved in Solvent Mixture: [Bufferl: Acetonitrile 90:10] and hence mobile phase is used as diluent

Various Method screening Trials has been taken using following different compositions.

➢ Table for trials:

Sr.No.	Trails Taken	Observation	Remarks
1	Buffer pH2.00 : Methanol (70:50 v/v), Flow rate 1.5 ml/min Column:- Symmetry C 18 150 X 4.6, 5µm	No Peak observed	Not Satisfactory
2	Buffer4.00 : ACN (70:50 v/v), Flow rate 1.5 ml/min Column:- Symmetry C 18 150 X 4.6, 5µm	No Peak observed	Not Satisfactory
3	Buffer: Acetonitrile (70:30 v/v), Flow rate 1.5 ml/min Column:- Symmetry C 18 150 X 4.6, 5µm	Peak observed	Not Satisfactory
4	Buffer: Acetonitrile : Methanol (80:10:10 v/v), Flow rate 2.0 ml/min Column:- Symmetry C 18 150 X 4.6, 5µm	Broaden peak observed	Not Satisfactory
5	Buffer: Acetonitrile : Methanol (90:05:05 v/v), Flow rate 1.8 ml/min Column:- Symmetry C 18 150 X 4.6, 5µm	Tailing observed	Not Satisfactory
6	Acetonitrile : Buffer (10:90 v/v), Flow rate 1.8 ml/min Column:- Symmetry C 18 150 X 4.6, 5µm	Good peak shape observed and separated from benzoic acid peak	Satisfactory

Reason for validation: Non-Pharmacopeial method.

Design of experiment (DOE):

A smart DOE was performed with respect to components of mobile phase (like concentration of buffering agent/ buffer strength, pH of buffer, ratio of buffer ad organic modifiers) and chromatographic parameter (like Flow rate and column temperature) as mentioned below.

1. Molarity of buffer Sodium Perchlorate conc. 1gm +/- 0.01g
2. Triethylamine conc. 1mL +/- 0.01mL
3. pH of buffer pH 4.3 +/- 0.2
4. Buffer ratio 900 mL +/- 90mL

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5. Acetonitrile 100 mL +/- 10mL
6. Flow rate 1.8 +/-0.2mL
7. Column temp 30+/- 5 °C

Method Validation:

- Standard preparation:

Weigh and transfer about 100 mg of Risatriptan (Rizatriptan Benzoate) reference standard to a 100 mL volumetric flask and dissolve and dilute up to the mark with mobile phase, further dilute 10mL solution to 100mL with mobile phase.

- Sample preparation

Weigh accurately not less than 20 tablet crush and weigh powder equivalent to 100mg of label amount into 100 mL volumetric flask add about 75 mL of mobile phase, sonicate at for about 15 min with intermittent shaking, keep achieve room temperature make up to volume with mobile phase, further dilute 5mL solution to 50mL with mobile phase.

- Buffer Preparation

Added 1 gm of sodium perchlorate and 1ml triethylamine in 1000ml water and adjust pH 4.30 with 10% Acetic acid.

- Mobile phase Preparation

Mix 100 ml of Acetonitrile and 900ml of buffer solution, sonicate and filter through 0.45μ membrane filter and degas.

- Diluent/Blank Solution:
  Use mobile phase as blank.
- Optimized HPLC Parameters:

Instrument	: Agilent Liquid Chromatography with PDA detector
Column	: Symmetry C-18, 150 mm x 4.6 mm, 5.0 μm
Flow Rate	: 1.8 mL/min
Injection volume	: 20 μL
Column temperature	: 30°C
Sample cooler Temperature	: Ambient
Detection	: 225 nm
Run time	: 15 minutes

System Suitability Test:

Sr. No.	Parameters	Risatriptan (Rizatriptan Benzoate)
1.	Peak area	*5670421*
2.	No. of theoretical plates	*8529*
3.	Retention time (min)	5.312
4.	Asymmetry/USP Tailing	1.02
5.	% RSD	*0.11*

Specificity:

Specificity Part-I: Interference from blank, benzoic acid and placebo

- Procedure

Prepare blank preparation, prepared placebo preparation, standard preparation, and sample preparation for 5 and 10mg tablet as per the method.

- Benzoic acid solution preparation

Transfer accurately measured quantity of acetic acid 50 mg and transferred to a 100 mL volumetric flask add about 75 mL of mobile phase , mix and make up to volume with mobile phase, further dilute 10mL to 100mL with mobile phase.

- Placebo preparation

Weighed accurately placebo equivalent to 100 mg of Risatriptan (Rizatriptan Benzoate) and transferred to a 100 mL volumetric flask add about 75 mL of mobile phase , sonicate at for about 15 min with intermittent shaking, keep to achieve room temperature make up to volume with mobile phase, further dilute 10mL to 100mL with mobile phase.

Observation: No interference seen

Specificity Part-II :Forced degradation

Sr. No.	Stress type	% Degradation	Observation
1	Untreated sample	----	No peak observed from the excipient blend at the retention time of Risatriptan (Rizatriptan Benzoate).
2	Heat degradation (Solid state)	NIL	No peak observed from the excipient blend at the retention time of Risatriptan (Rizatriptan Benzoate)
3	Heat degradation (Solution state)	NIL	No peak observed from the excipient blend at the retention time of Risatriptan (Rizatriptan Benzoate)
4	Photolytic degradation	NIL	No peak observed from the excipient blend at the retention time of Risatriptan (Rizatriptan Benzoate)
5	Humidity degradation	NIL	No peak observed from the excipient blend at the retention time of Risatriptan (Rizatriptan Benzoate)
6	Acid dégradation	5.86%	No peak observed from the excipient blend at the retention time of Risatriptan (Rizatriptan Benzoate)
7	Base degradation	10.15%	No peak observed from the excipient blend at the retention time of Risatriptan (Rizatriptan Benzoate)

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Sr. No.	Stress type	% Degradation	Observation
8	Peroxide degradations	16.53%	No peak observed from the excipient blend at the retention time of Risatriptan (Rizatriptan Benzoate)

Linearity and Range:

Linearity Level	Standard concentration	Concentration of Risatriptan (Rizatriptan Benzoate) (ppm)	Mean area (n = 3)	Regression coefficient (R^2)
Level – 1	50%	50.20	2952527	0.9997
Level – 2	80%	75.30	4051177	
Level – 3	100%	100.40	5405053	
Level – 4	120%	125.50	6390534	
Level – 5	150%	150.60	7457580	

Precision:

Sample Preparation	% Assay of Risatriptan (Rizatriptan Benzoate)
Test solution -1	99.67
Test solution -2	99.59
Test solution -3	99.55
Test solution -4	99.38
Test solution -5	99.48
Test solution -6	99.69
Mean	99.56
Standard Deviation	0.12
Relative Standard Deviation (%)	0.12

Intermediate precision:

Analysis performed during method precision study Analyst: Analyst-I Make :Symmetry,C18, 4.6mmx150mm, 5 µm Column serial number. : 0402471K	HPLC ID No.: EAR040
Sr. No.	% Assay of Risatriptan (Rizatriptan Benzoate)
Test solution-1	99.67
Test solution-2	99.59
Test solution-3	99.55
Test solution-4	99.38
Test solution-5	99.48
Test solution-6	99.69
Analysis performed during intermediate precision study HPLC ID No.: EAR039 Make :Symmetry,C18, 4.6mmx150mm, 5 µm Column serial number : 0502481L	
Test solution-1	99.44
Test solution-2	99.56
Test solution-3	100.05
Test solution-4	99.42
Test solution-5	99.64
Test solution-6	99.83
Mean of twelve samples	99.66
Standard Deviation	0.24
Relative Standard Deviation (%)	0.24

Robustness:

- Change the flow rate of Mobile Phase:

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Parameter	Test solution	%Assay for Risatriptan (Rizatriptan Benzoate)
Method precision	1	*99.67*
	2	*99.59*
	3	*99.55*
	4	*99.38*
	5	*99.48*
	6	*99.69*
Change in flow rate 1.6 mL/ min.	1	99.65
	2	99.38
Mean		99.55
Standard deviation		0.12
Relative standard deviation (%)		0.12

Parameter	Test solution	%Assay for Risatriptan (Rizatriptan Benzoate)
Method precision	1	*99.67*
	2	*99.59*
	3	*99.55*
	4	*99.38*
	5	*99.48*
	6	*99.69*
Change in flow rate 2.0 mL/ min.	1	99.21
	2	98.99
Mean		99.44
Standard deviation		0.24
Relative standard deviation (%)		0.24

- Change in the Mobile Phase composition $\pm$10%:

Parameter	Test solution	%Assay for Risatriptan (Rizatriptan Benzoate)
Method precision	1	99.67
	2	99.59
	3	99.55
	4	99.38
	5	99.48
	6	99.69
Change in organic component +10%	1	99.78
	2	99.52
Mean		99.55
Standard deviation		0.13
Relative standard deviation (%)		0.13

Parameter	Test solution	%Assay for Risatriptan (Rizatriptan Benzoate)
Method precision	1	99.67
	2	99.59

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	3	99.55
	4	99.38
	5	99.48
	6	99.69
Change in organic component -10%	1	99.11
	2	99.68
Mean		99.52
Standard deviation		0.20
Relative standard deviation (%)		0.20

➢ Change in the Temperature of the Column ±5°C:

Parameter	Test solution	%Assay for Risatriptan (Rizatriptan Benzoate)
Method precision	1	99.67
	2	99.59
	3	99.55
	4	99.38
	5	99.48
	6	99.69
Change in Temperature of Column oven +5°C	1	99.05
	2	100.65
Mean		99.63
Standard deviation		0.46
Relative standard deviation (%)		0.46

Parameter	Test solution	%Assay for Risatriptan (Rizatriptan Benzoate)
Method precision	1	99.67
	2	99.59
	3	99.55
	4	99.38
	5	99.48
	6	99.69
Change in Temperature of Column oven -5°C	1	101.01
	2	100.65
Mean		99.88
Standard deviation		0.60
Relative standard deviation (%)		0.60

➢ Change in buffer component of mobile phase ±10%:

Parameter	Test solution	%Assay for Risatriptan (Rizatriptan Benzoate)
Method precision	1	99.67
	2	99.59
	3	99.55
	4	99.38

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	5	*99.48*
	6	*99.69*
Change in Buffer component +10%	1	*100.25*
	2	*100.01*
Mean		*99.70*
Standard deviation		*0.29*
Relative standard deviation (%)		*0.29*
Parameter	Test solution	%Assay for Risatriptan (Rizatriptan Benzoate)
Method precision	1	*99.67*
	2	*99.59*
	3	*99.55*
	4	*99.38*
	5	*99.48*
	6	*99.69*
Change in Buffer component -10%	1	*99.65*
	2	*100.15*
Mean		*99.65*
Standard deviation		*0.23*
Relative standard deviation (%)		*0.23*

- System suitability parameters:

Parameter			Theoretical Plates	Tailing Factor	%RSD
Limits			Not less than 2500	Not more than 2.0	Not more than 2.0%
1	Specificity				
	1.1	Specificity-Part-A	8529	1.02	0.11
	1.2	Specificity-Part-B	8313	1.05	0.15
2	Linearity and Range		8204	1.02	0.18
3	Accuracy study (Recovery)		8735	1.02	0.23
4	Precision				
	4.1	Method precision (Repeatability)	8526	1.04	0.12
	4.2	Intermediate Precision (Ruggedness)	8431	1.06	0.24
5	Robustness				
	5.1	Change flow rate by ± 10% (1.6 ml/minute and 2.0 ml/minute).	7952 8358	1.09 1.24	0.12 0.47
	5.2	Change the column temperature by ± 5°C (25°C and 35°C)	7952 8878	1.08 1.01	0.62 0.19
	5.3	Change the mobile phase Organic components by ± 10%	7952 6754	1.09 1.11	0.58 0.39
	5.4	Change the mobile phase Buffer components by ± 10%	7952 8886	1.09 1.31	0.58 0.79

Reason for validation: Non-Pharmacopeial method

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The results of analysis in terms of % label claim were found to be 98.00 to 102.00 for formulation analyzed.
Chromatograms:

Auto-Scaled Chromatogram

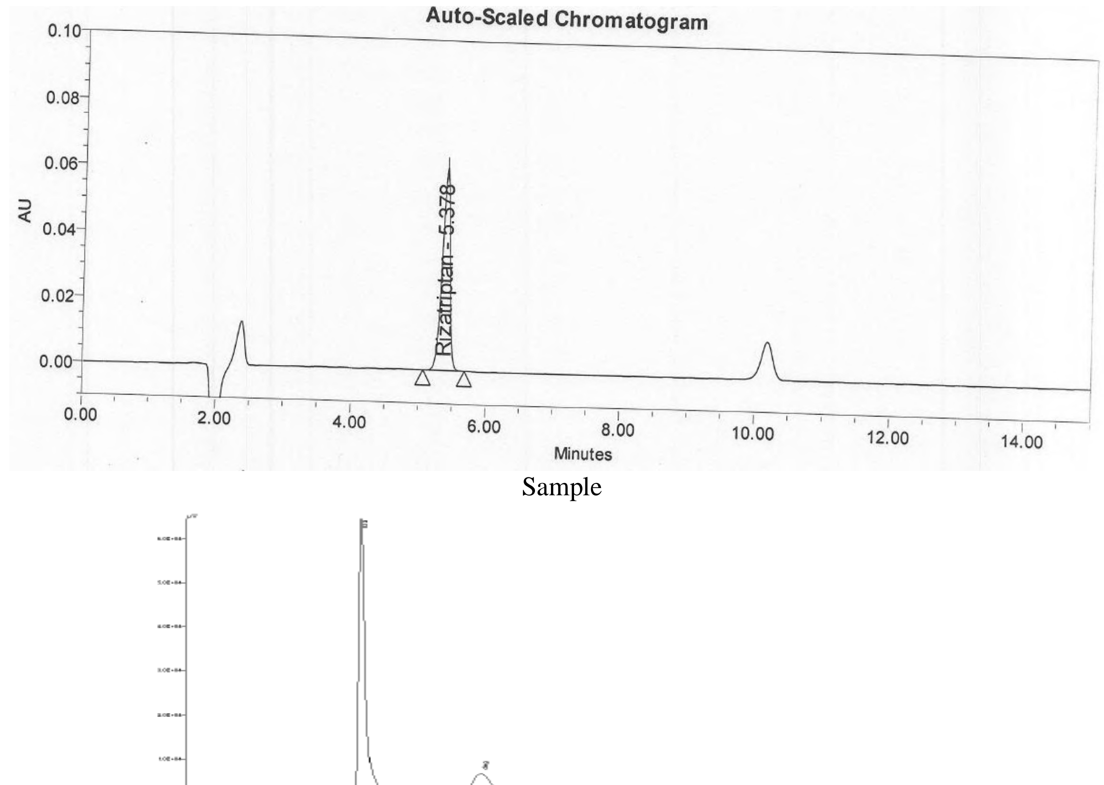
Blank

Auto-Scaled Chromatogram

Placebo

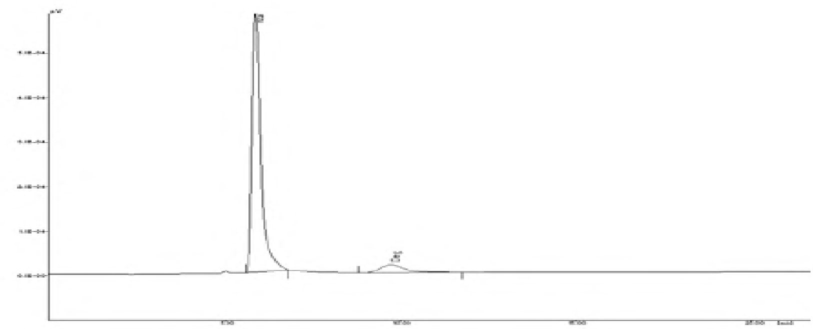
Auto-Scaled Chromatogram

Standard

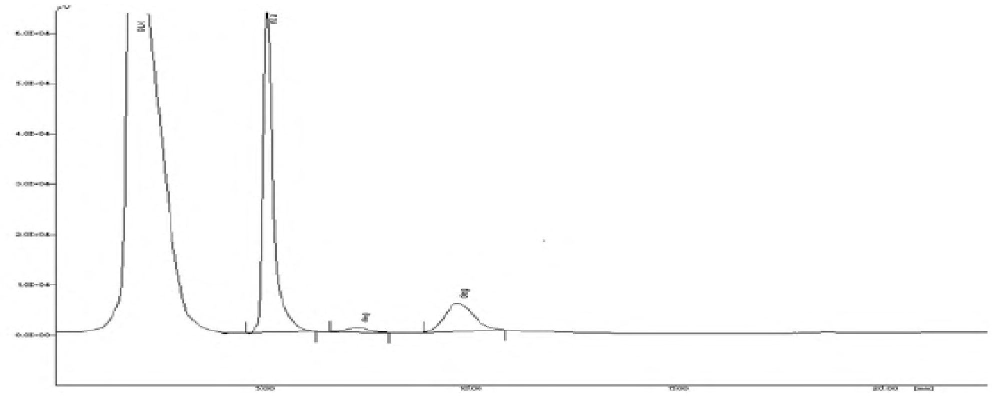


Sample

Chromatogram of Base degradation sample for Risatriptan (Rizatriptan Benzoate) tablet



Chromatogram of Acid degraded sample for Risatriptan (Rizatriptan Benzoate) tablet



Chromatograms of per oxidation degraded sample for Risatriptan (Rizatriptan Benzoate) tablet

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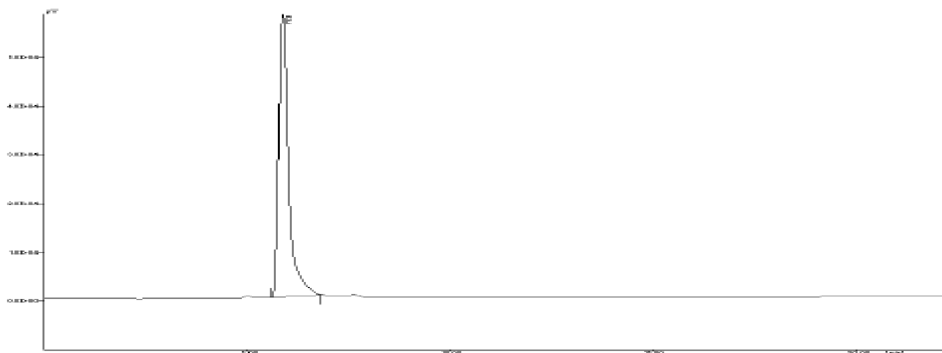
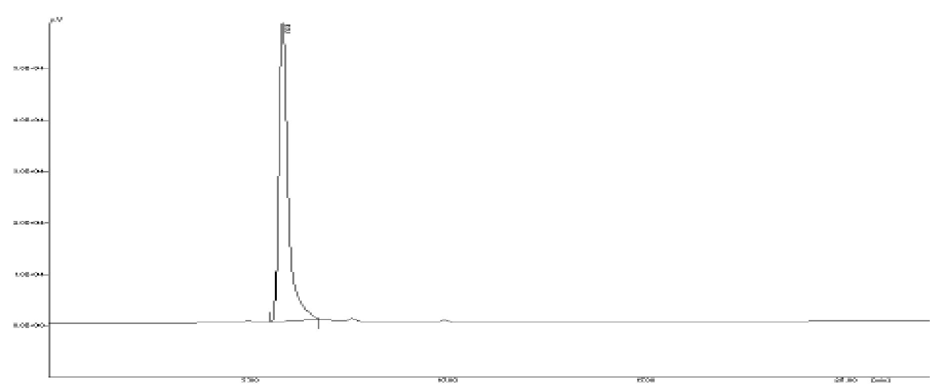


Chromatograms of thermal-degraded-Solution state sample for Risatriptan (Rizatriptan Benzoate) tablet

Chromatograms of photo-degraded sample for Risatriptan (Rizatriptan Benzoate) tablet



Chromatograms of Humidity degradation sample for Risatriptan (Rizatriptan Benzoate) tablet

Result and Discussion:

- Specificity Part-I

There is no interference of blank and placebo peaks with the main peak. All impurities are well separated from the main peak. The main peak purity and known impurities purity is well within the limit of acceptance criteria. The results obtained are well within acceptance criteria. Hence the method can be termed as specific.

- Specificity Part-II
  - Degraded impurities in all sample preparation are well separated from the main peak.
  - Peak purity for the main peak in sample preparation is well within the limit of acceptance criteria.

Hence the method can be termed as specific

- Linearity and Range

The areas obtained are directly proportional to the concentration of analyte in the sample. Hence the method considered as linear in the range considered.

- Accuracy

The recovery at each level and mean recovery meets the established acceptance criteria.
Hence, the method can be termed as accurate in the considered range.

- Precision

The results obtained lie well within the limit of acceptance criteria. Hence the method can be termed as precise and rugged.

- Filter media interference

The results obtained lie well within the limit of acceptance criteria. Hence there is no interference from filter media.

- Robustness

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No significant changes observed in system suitability parameters.
Hence, the method can be termed as robust.

- System Suitability

The mean values of system suitability parameters are well within acceptance criteria, hence the method is suitable
Since the results are within the limit of acceptance criteria for all validation parameters, therefore, the method can be considered as validated and suitable for intended use.

Conclusion:
The proposed method for determination of Risatriptan (Rizatriptan Benzoate) is simple, specific, rapid, linear, accurate, precise, rugged, robust, sensitive as well as selective and suitable for routine analysis in laboratories.

Acknowledgement:
I am thankful to my guide Dr. Rahul V. Mayee, Dr. Ajit Ekal, Managing Director, Instavision laboratory and Services, Satara for their technical help in this project. I am also thankful to our Principal YSPM's YTC, Faculty of Pharmacy Dr. V. K. Redasani and Management for their motivation and support.

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Life Cycle Management of Analytical RP-HPLC Method Development for Assay of Abilify Disc melts in Immediate Release Dosage Form

Mr. Avinash M. Bhagwat[1]
Dr. Rahul V. Mayee[2]
Dr. Ajit B. Ekal[3]

[1]Assistant Professor, YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara
[2]Head MW & RA, BioRadius Therapeutic Research Pvt. Ltd. Pune
[3]Managing Director, Instavision laboratory and Services, Satara

Abstract: In this research work of life cycle management of analytical RP-HPLC method development for assay of Ability Disc melt in immediate release dosage form, the RP-HPLC assay method was developed and validated for Ability Disc melt. Stress study is also carried out. The chromatographic conditions will be as, mobile phase is 0.05M Phosphate buffer pH2.0, Methanol, Acetonitrile in a ratio of 5:3:2, column used is Hypersil ODS C-18, 250 mm x 4.6 mm, 5.0 µm, Flow Rate1.5 ml/min, Injection volume 20 µl, Detection wavelength is 215 nm and run time is 25 min. This life cycle management stability indicating RP-HPLC analytical method is economical, specific, accurate, precise and robust for assay of Ability Disc melt in immediate release dosage form.

Key words: Abilify Discmelt, Life Cycle Management, RP-HPLC Method Development, Validation, Immediate Release Dosage Form

Introduction:
Abilify Disc melt (Aripiprazole)) is a typical antipsychotic agent, which is used in a treatment of schizophrenia, bipolar I disorder and acute treatment of manic and mixed episodes [1-3]. It is also used in Tourette's disorder in pediatrics patients (16-18 years) in the dose range 5-20 mg/day patient weight less than 50 kg. It has chemical name 7-(-4-(4-2-3- dichlorophenyl)-1-piperazinyl)butoxy)-3,4-dihydrocarbostyr il (Figure 1). It is effective in the treatment of both negative and positive symptoms of schizophrenia disorder. This agent belongs to the class of benzioxazole with dose 10-15 mg/day. It has partial agonist effect towards 5- HT1A receptor, dopamine D2 receptor and antagonistic effect on 5- HT2 receptor. Its sides effects including weight gain, QTc prolongation and hyperprolactinemia [4]. On the basis of literature survey few analytical methods reported for the detection of Aripiprazole in pharmaceutical dosage forms and biological fluids include high performance liquid chromatography (HPLC), gas chromatography-mass spectroscopy (GC-MS), liquid chromatography-tandem mass spectroscopy (LC-MS/MS), capillary electrophoresis and spectrophotometric methods have been describe for the determination of Aripiprazole in pharmaceutical preparations [5,7]. The purpose of the current study was to develop a validated HPLC method for determination of Aripiprazole in bulk and pharmaceutical dosage form, to achieve more accuracy, specificity and precision. The method validation was preformed according to ICH guidelines. The method designed for estimation of Aripiprazole is more superior than previously reported methods and water is used as major part of solvents and less use of hazardous organic solvents.

Structure of Abilify Discmelt (Aripiprazole)

Experimental:

- **Materials:**

1) Abilify Discmelt (Aripiprazole): Working standard and its claimed purity was 98.20%.
2) Abilify Discmelt (Aripiprazole): Tablet (label claim 10mg) and placebo, which was prepared and supplied by Instavision lab,.

Note: No any known Impurity reported.

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- **Reagents and Chemicals:**

1) Acetonitrile: -HPLC grade, Rankem, India.
2) Methanol: - HPLC grade, Rankem, India.
3) Milli-Q water: - It was purified by Millipore Corporation’s system.
4) Acetic acid: - Reagent Grade, Merck, India.
5) Hydrochloric acid: - Reagent Grade, Merck, India.
6) Sodium hydroxide: - Reagent Grade, Merck, India.
7) Hydrogen Peroxide (30%):- Reagent Grade, Merck, India.
8) Potassium di-hydrogen orthophosphate - AR Grade, Merck, India.

- **Instruments, Apparatus and equipment:**

1) High Performance Liquid chromatography system (HPLC): Agilent Liquid Chromatography with PDA detector
2) Chromatographic software:– E Z Chrome Elite
3) A double beam UV-visible spectrophotometer having two matched cells with 1cm light path: - UV- 2450, Shimadzu, Japan.
4) Analytical Balance: - AD 265S, Mettler Toledo, Sweetzerland.
5) pH Meter: - Labindia, India.
6) Sonicator: - 5510, Branson Ultrasonics Corporation, Danbury, CT, USA.
7) Hot air oven: - Labline, India.
8) Photo stability chamber: - SVI equipment’s, Germany

- **Chromatographic system:**

Degradation studies were carried out on a system consisted of 1200 series HPLC (Agilent Technologies) comprising of an on-line degasser (G1322A), binary pump (G1312A), auto injector (G1367C), column oven (G1310B), DAD detector (G1315C) and E Z Crome Elite (software).The published methods of analysis for determination and separation of Abilify Discmelt (Aripiprazole) in their formulation were not evaluated for specificity and degradation study. Therefore, method having specificity for degradation products and formulation excipients is considered as a prime requirement. Degraded samples, prepared by systematic forced degradation study, were used for method development trials to optimize the method as a stability indicating method for determination of Abilify Discmelt (Aripiprazole) .

- **Selection of Buffer in Mobile Phase: -**

0.05M Phosphate buffer pH2.0 with orthophosphoric acid was used to optimize the peak shape retention time and to proper separation of impurities peaks from main drugs peaks. The ratio of (Buffer: Methanol: Acetonitrile) was selected on the basis of resolution between the major degradation peaks and main peaks, and it was finalized as (50:30:20) v/v after analyzing all the degraded samples and evaluating the peak purity, resolution, specificity and stability indicating nature of the method.

- **Selection of Mobile Phase: -**

Different ratios of Acetonitrile and Methanol was used to optimize the retention time of late eluting impurities and Methanol to proper separation of impurities peaks from main drugs peaks. The ratio of (Buffer: Methanol: Acetonitrile) was selected on the basis of resolution between the major degradation peaks and main peaks, and it was finalized as (Buffer: Methanol: Acetonitrile) [50:30:20 v/v] after analyzing all the degraded samples and evaluating the peak purity, resolution, specificity and stability indicating nature of the method.

- **Selection of Column:-**

For HPLC, various columns are available, but as the main aim of the method to resolve the compound in the presence of polar and non-polar degradation products and impurities, a C_{18} column was preferred over other columns Hypersil ODS C-18, 250 mm x 4.6 mm, 5 µm column was chosen to give good peak shape, good lifetime and high resolution on compared to other C_{18} columns.

- **Selection of Diluents / Solvent for extraction:-**

Different solvents were tried including single solvent and combination of solvents like ACN: Water, Methanol: Water, in different concentrations, But Abilify Discmelt (Aripiprazole) tablet gets dissolved in Methanol. Hence first stock was prepared in methanol and followed by second dilution done in diluents as [Methanol: Acetonitrile: Buffer 30:20:50] same as that of mobile phase to reduce the peak shape related problems.

The results of all validation parameters are given in following tables and all lie well within the limit of acceptance criteria, Various Method screening Trials has been taken using following different compositions.

- **Table for trials:**

Sr. No.	Trails Taken	Observation	Remarks
1	Buffer : Methanol (50:50 v/v), Flow rate 1.0 ml/min Column:- Hypersil ODS C 18 250 X 4.6, 5µm	No Peak observed	Not Satisfactory
2	Buffer : Acetonitrile (50:50 v/v), Flow rate 1.0 ml/min Column:- Hypersil ODS C 18 250 X 4.6, 5µm	Peak observed later.	Not Satisfactory
3.	Buffer : Acetonitrile : Methanol (50:25:25 v/v), Flow rate 2.0 ml/min Column:- Hypersil ODS C 18 250 X 4.6, 5µm	Broaden peak observed	Not Satisfactory
4	Buffer : Acetonitrile : Methanol (50:25:25 v/v), Flow rate 1.5 ml/min Column:- Hypersil ODS C 18 250 X 4.6, 5µm	Tailing observed	Not Satisfactory
5	Buffer : Acetonitrile : Methanol (50:20:30 v/v), Flow rate 1.5 ml/min Column:- Hypersil ODS C 18 250 X 4.6, 5µm	Good peak shape observed	Satisfactory

Reason for validation: Non-Pharmacopeia method.

Design of experiment (DOE):
A smart DOE was performed with respect to components of mobile phase (like concentration of buffering agent/ buffer strength, pH of buffer, ratio of organic modifiers) and chromatographic parameter (like Flow rate and column temperature) as mentioned below.

1. KH_2PO_4 conc. 0.05M +/- 0.01
2. pH of buffer pH 2.0 +/- 0.2
3. Buffer ratio 500 mL +/- 50mL
4. Methanol 300 mL +/- 30mL
5. Acetonitrile 200 mL +/- 20mL
6. Flow rate 1.5 +/-0.2mL
7. Column temp 40+/- 5 °C

Method Validation:

- **Standard preparation:**

Weigh and transfer about 30 mg of Abilify Discmelt (Aripiprazole) reference standard to a 200 mL volumetric flask. Add about 140 mL of Methanol, sonicate to dissolve, make up the volume with solvent Methanol. Further dilute 10 mL of this solution and make up the volume 25 mL with mobile phase. (60 ppm)

- **Sample preparation**

Weigh accurately tablet powder equivalent to 15mg transfer into 100 mL volumetric flask add about 75 mL of methanol , sonicate at for about 45 min with intermittent shaking, keep to achieve room temperature make up to volume with methanol. Centrifuge the solution at 3500rpm for 20 minutes and further dilute 10mL of the supernatant to 25mL with mobile phase. (60 ppm)

- **Mobile phase Preparation**

Mixture of (Buffer:- 0.05M Phosphate buffer pH2.0) 500 mL buffer, 300 mL methanol, 200 mL Acetonitrile and filter through 0.45µ membrane filter and degas.

- **Blank Solution:**
  Use mobile phase as blank.

- **Optimized HPLC Parameters:**

Instrument : Agilant Liquid Chromatography with PDA detector
Column : Hypersil ODS C-18, 250 mm x 4.6 mm, 5.0 µm
Flow Rate : 1.5 mL/min
Injection volume : 20 µL
Column temperature : 40°C
Sample cooler Temperature : Ambient

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Detection : 215 nm
Run time : 25 minutes

System Suitability Test:

Sr. No.	Parameters	Abilify Discmelt (Aripiprazole)
1.	Peak area	*1183271*
2.	No. of theoretical plates	*8529*
3.	Retention time (min)	8.212
4.	Asymmetry/USP Tailing	1.02
5.	% RSD	*0.35*

Linearity:

Linearity Level	Standard concentration	Concentration of Abilify Discmelt (Aripiprazole) (ppm)	Mean area (n = 3)	Regression coefficient (R^2)
Level – 1	50%	30.08	592833	0.9999
Level – 2	80%	48.12	946507	
Level – 3	100%	60.15	1186807	
Level – 4	120%	72.18	1425522	
Level – 5	150%	90.23	1765958	

Precision:

Sample Preparation	% Assay of Abilify Discmelt (Aripiprazole)
Test solution -1	99.57
Test solution -2	99.49
Test solution -3	99.40
Test solution -4	99.28
Test solution -5	99.59
Test solution -6	99.39
Mean	**99.45**
Standard Deviation	**0.12**
Relative Standard Deviation (%)	**0.12**

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Intermediate precision:

Analysis performed during method precision study

Analyst: Analyst-I HPLC ID No.: ASR34

Make :Hypersil ODS,C18, 4.6mmx250mm, 5 µm
Column serial number. : 12058H

Sr. No.	% Assay of Abilify Discmelt (Aripiprazole)
Test solution-1	99.57
Test solution-2	99.49
Test solution-3	99.40
Test solution-4	99.28
Test solution-5	99.59
Test solution-6	99.39

Analysis performed during intermediate precision study
HPLC ID No.: ASR34
Make :Hypersil ODS,C18, 4.6mmx250mm, 5 µm
Column serial number : 05482J

Test solution-1	99.64
Test solution-2	99.53
Test solution-3	100.02
Test solution-4	99.32
Test solution-5	99.62
Test solution-6	99.82
Mean of twelve samples	**99.56**
Standard Deviation	**0.21**
Relative Standard Deviation (%)	**0.21**

Robustness:

- **Change the flow rate of Mobile Phase:**

Parameter	Test solution	%Assay for Abilify Discmelt (Aripiprazole)
Method precision	1	99.57
	2	99.49
	3	99.40
	4	99.28
	5	99.59
	6	99.39
Change in flow rate 1.30 mL/ min.	1	99.55
	2	99.30
Mean		**99.45**
Standard deviation		**0.12**
Relative standard deviation (%)		**0.12**
Parameter	**Test solution**	**%Assay for Abilify Discmelt (Aripiprazole)**
Method precision	1	99.57
	2	99.49
	3	99.40

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	4	99.28
	5	99.59
	6	99.39
Change in flow rate 1.70 mL/ min.	1	99.11
	2	98.95
Mean		**99.35**
Standard deviation		**0.22**
Relative standard deviation (%)		**0.22**

- **Change in the Mobile Phase composition +10%:**

Parameter	Test solution	%Assay for Abilify Discmelt (Aripiprazole)
Method precision	1	99.57
	2	99.49
	3	99.40
	4	99.28
	5	99.59
	6	99.39
Change in Mobile Phase composition +10% {higher organic- Buffer: MeOH: ACN) (500:330:220)}	1	100.14
	2	100.69
Mean		99.69
Standard deviation		0.48
Relative standard deviation (%)		0.48
Parameter	**Test solution**	**%Assay for Abilify Discmelt (Aripiprazole)**
Method precision	1	99.57
	2	99.49
	3	99.40
	4	99.28
	5	99.59
	6	99.39
Change in Mobile Phase composition - 10%. {Buffer:MeOH:ACN) (500:270:180)}	1	99.98
	2	101.01
Mean		99.71
Standard deviation		0.56
Relative standard deviation (%)		0.57

- **Change in the Temperature of the Column +5°C:**

Parameter	Test solution	%Assay for Abilify Discmelt (Aripiprazole)

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Method precision	1	99.57
	2	99.49
	3	99.40
	4	99.28
	5	99.59
	6	99.39
Change in Temperature of the Column +5°C	1	99.66
	2	99.87
Mean		99.53
Standard deviation		0.18
Relative standard deviation (%)		0.19
Parameter	**Test solution**	**%Assay for Abilify Discmelt (Aripiprazole)**
Method precision	1	99.57
	2	99.49
	3	99.40
	4	99.28
	5	99.59
	6	99.39
Change in Temperature of the Column -5°C	1	100.14
	2	99.68
Mean		99.57
Standard deviation		0.26
Relative standard deviation (%)		0.27

➢ **Change in the pH of the Buffer ±0.1Unit:**

Parameter	**Test solution**	**%Assay for Abilify Discmelt (Aripiprazole)**
Method precision	1	99.57
	2	99.49
	3	99.40
	4	99.28
	5	99.59
	6	99.39
Change in pH of the buffer by + 0.1 unit	1	101.01
	2	100.65
Mean		99.80
Standard deviation		0.65
Relative standard deviation (%)		0.65
Parameter	**Test solution**	**%Assay for Abilify Discmelt (Aripiprazole)**
Method precision	1	99.57

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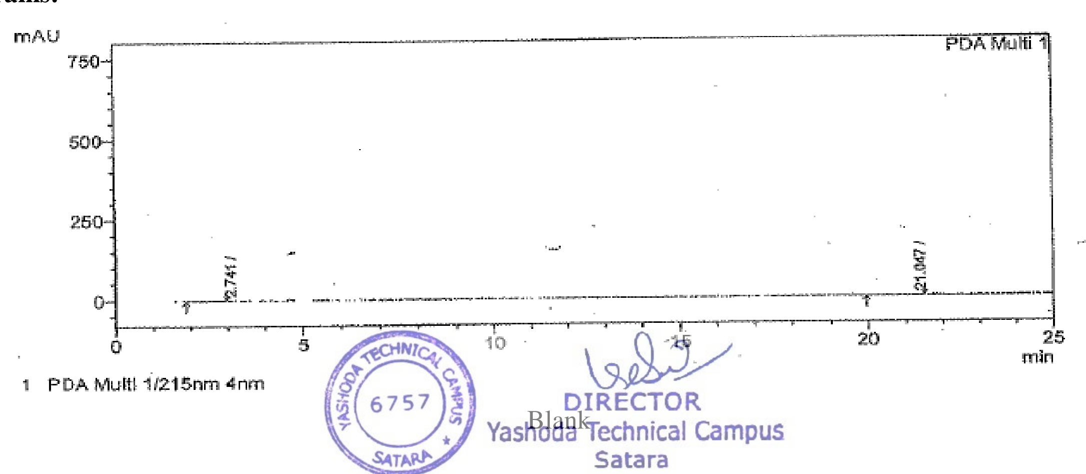
	2	99.49
	3	99.40
	4	99.28
	5	99.59
	6	99.39
Change in pH of the buffer by - 0.1 unit	1	99.98
	2	100.15
Mean		99.61
Standard deviation		0.30
Relative standard deviation (%)		0.31

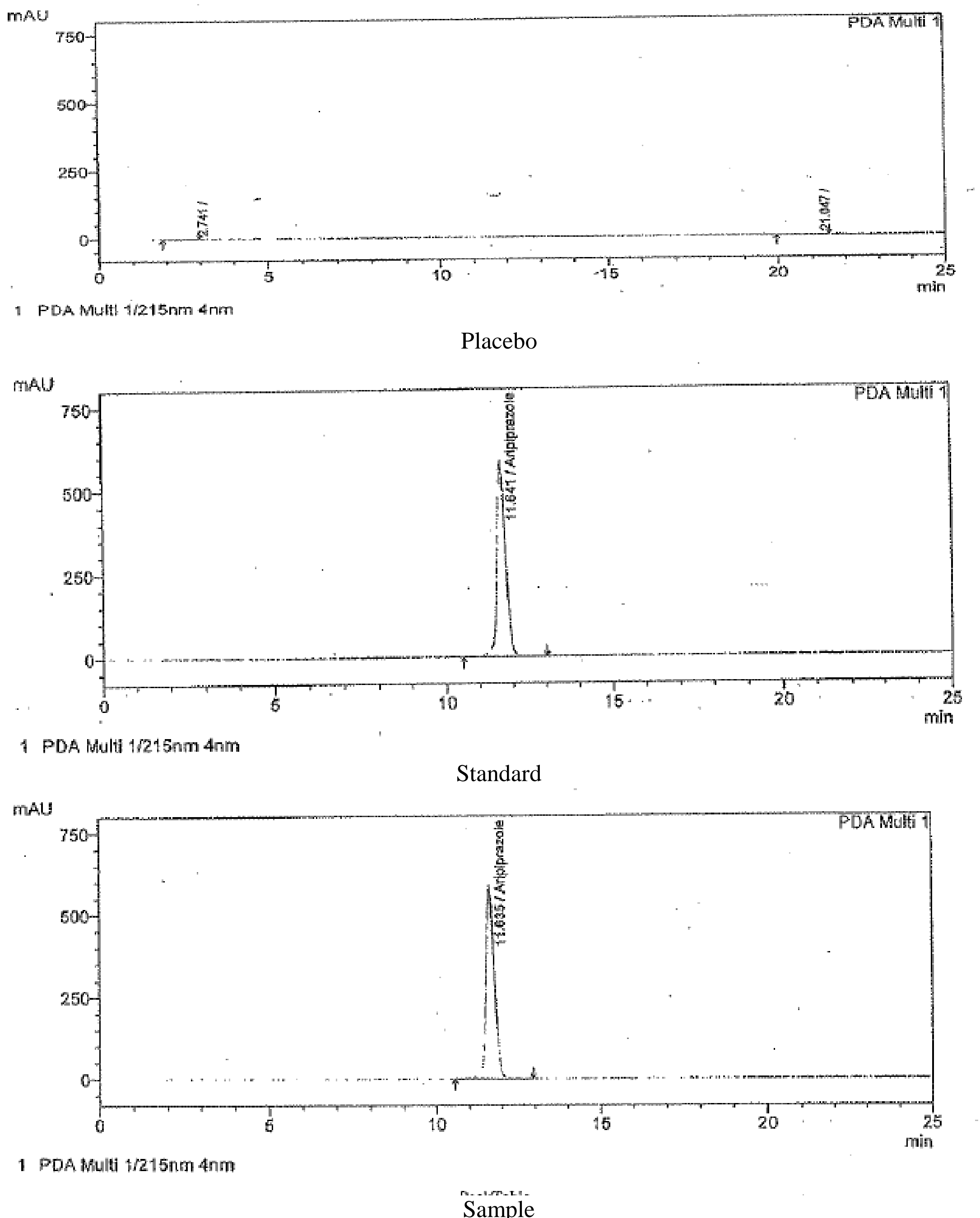
➢ **System suitability parameters:**

		Parameter	Theoretical Plates	Tailing Factor	%RSD
		Limits	Not less than 2500	Not more than 2.0	Not more than 2.0%
1	**Specificity**				
	1.1	Specificity-Part-A	8952	1.07	0.13
	1.2	Specificity-Part-B	8213	1.05	0.15
2	**Linearity and Range**		8201	1.03	0.17
3	**Accuracy study (Recovery)**		8135	1.02	0.16
4	**Precision**				
	4.1	Method precision (Repeatability)	8546	1.04	0.14
	4.2	Intermediate Precision (Ruggedness)	8451	1.06	0.12
5	**Robustness**				
	5.1	Change flow rate by ± 10% (1.3 ml/minute and 1.8 ml/minute).	8212 8672	1.09 1.01	0.60 0.46
	5.2	Change the column temperature by ± 5°C (35°C and 45°C)	8014 7753	1.08 1.28	0.60 0.85
	5.3	Change the mobile phase Organic components by ± 10%	7845 8911	1.09 1.20	0.68 1.02
	5.4	Change the mobile phase Buffer pH by ± 0.1 Unit	8162 6785	1.11 1.29	0.57 1.36

Chromatograms:



Blank



Placebo

Standard

Sample

Result and Discussion:

- **Specificity Part-A**

There is no interference of blank and placebo peaks with the main peak. All impurities are well separated from the main peak. The main peak purity and known impurities purity is well within the limit of acceptance criteria. The results obtained are well within acceptance criteria. Hence the method can be termed as specific.

- **Specificity Part-B**
  - Degraded impurities in all sample preparation are well separated from the main peak.
  - Peak purity for the main peak in sample preparation is well within the limit of acceptance criteria.
  - Hence the method can be termed as specific

- **Linearity and Range**

The areas obtained are directly proportional to the concentration of analyze in the sample. Hence the method considered as linear in the range considered.

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- **Accuracy**
  The recovery at each level and mean recovery meets the established acceptance criteria. Hence, the method can be termed as accurate in the considered range.

- **Precision**
  The results obtained lie well within the limit of acceptance criteria. Hence the method can be termed as precise and rugged.

- **Filter media interference**
  The results obtained lie well within the limit of acceptance criteria. Hence there is no interference from filter media.

- **Robustness**
  No significant changes observed in system suitability parameters. Hence, the method can be termed as robust.

- **System Suitability**

The mean values of system suitability parameters lay well within acceptance criteria, hence the method is suitable. Since the results are within the limit of acceptance criteria for all validation parameters, therefore, the method can be considered as validated and suitable for intended use.

Conclusion:
The proposed method for determination of Abilify Discmelt (Aripiprazole) is simple, specific, rapid, linear, accurate, precise, rugged, robust, sensitive as well as selective and suitable for routine analysis in laboratories.

Acknowledgement:
I am thankful to my guide Dr. Rahul V. Mayee, Dr. Ajit Ekal, Managing Director, Instavision laboratory and Services, Satara for their technical help in this project. I am also thankful to our Principal YSPM's YTC, Faculty of Pharmacy Dr. V. K. Redasani and Management for their motivation and support.

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FORMULATION AND EVALUATION OF ALOEVERA AND VITAMIN E PEEL OF MASK

TAYAPPA BM*, DEVALE RP, CHAWARE VJ AND REDASANI VK

YSPM's Yashoda Technical Campus, Faculty of Pharmacy NH-4, Wadhe, Satara, M.S., India

***Corresponding Author: Bansode Mira Tayappa: E Mail: mirabansode96@gmail.com**

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ABSTRACT

Peel off mask is the type of dosage form which is gently applied onto the facial skin surface and is peeled off after few minutes of its application. It is used as the remedy to treat facial skin related problems such as wrinkles, ageing, acne and mainly used to open the closed pores due to deposition of dust. Aloe vera and Vitamin E is being added as an active ingredient in this formulation. Dosage formulations of peel of mask made in type of three methods. Further preparation peel of mask evaluated physical properties (organoleptic, homogenicity, P^H, spredability test, peeling time, irritation test). The results showed that the first method shows most of the evaluation parameters compatible during 2 weeks of storage.

Keyword: Aloe vera, Cosmetic, Peel of mask, Antioxidant

INTRODUCTION

Peel of face mask makes skin healthy People make many attempts to get beautiful and fresher skin. But due to dust, pollution, unhealthy eating habits and poor daily routine, many skin related problems erupt. People use beauty products and home remedies to avoid these problems. In this regard, a peel-off mask can also be a better option as it removes the skin dirt from the inside and also destroys dead skin cell. The peel-off mask also reduces many skin problems and keeps the skin healthy and fresh **[1]**.

Applying a face mask and using cosmetics to maintain beauty has been a beauty ritual practiced since ancient times. In fact, we know that the first cosmetic product ever made was a face mask. Face masks can nourish, cleanse, moisturize and tone the

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skin while also providing essential active ingredients for skin care **[2]**.

Indian women prepare different kinds of face masks at home. You can find many ready-made Ubtan powders on the market where you simply need to mix them with water or milk and apply them to your face. Ayurveda beauty care has not changed much since its introduction some five thousand years ago, proof of its effectiveness, safety and ease of use.

Roman women regularly used face masks as part of their beauty routine. Oils, honey, vinegar, basil juice and goose fat were popular ingredients. They also used some rather exotic ingredients such as placentas or stools of animals like kingfishers and cows **[3]**.

The pale look continued to be popular well into the times of Elizabeth I. By now, women had resorted to even more dangerous means to reach their desired skin color. They used hitherto unknown toxins such as white lead mixed with honey and olive oil to whiten their skin. But even this was not enough for some, and the practice of bloodletting continued. Fortunately, less invasive methods weren't completely discarded. Face masks made of egg whites and lemon juice were also used by some to brighten and nourish the complexion and achieved much safer results.Face masks in particular are available in all different types: creams, gels, powders or sheets. There are masks that choose to follow a more scientific and "chemical" approach and others that are rooted in a natural and holistic approach to skin care **[4]**.

MATERIALS AND METHODS

Carbopol 934 grade dissolved in water for 24 hr and after swell the carbopol is continuous stir with mechanical stirrer. Polyvinyl alcohol dissolved in warm water in water bath gently with continuous mechanical stirring and allows swelling for 2-3 hours then add methyl paraben. To this Disodium edetate (Solubilised in 1M NaOH) were loaded gently & dissolved. Drug solution along with carbopol and PVP were added slowly in swelled NaCMC (Sodium-carboxymethylcellulose) under continuous stirring. Citric acid added to the obtained solution to maintain PH and added in propylene glycol. Talcum powder mixed it to give the formulation opacity. Aloe extract dissolved in Rose water and added the vitamin E in continuous stirring. Final volume was made up with the purified water. After addition of whole ingredient, stirred continuously until a smooth dispersion obtained. Prepared formulation filled in collapsible tube for further analysis **[5, 6]**.

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Table 1: Ingredients for the Peel of Mask

Sr. No.	Ingredients	Manufactures
1.	Disodium edentate	S.D. FINE MUMBAI
2.	Polyvinyl Alcohol [PVP]	
3.	Carbopol [934grade]	
4.	Sodium carboxymethylcellulose	
5.	Methyl paraben	
6.	Propylene glycol	
7.	Talcum powder	
8.	Citric acid	
9.	Aloe extract	
10.	Vitamin E	
11.	Rose water	
12.	Water	

Figure 1: Mechanical stirrer

RESULT

The Aloe vera and vitamin E peel off mask was found to successful with good results. The peel off mask showed a good spredability. The formulation showed a good peel off property on human skin without causing skin irritation. The formulation showed good stability results and was found to be stable till room temperature.

The entire required ingredient to prepare peel of mask Aloe vera powder and vitamin E from the aloe Vera powder extract and vitamin E capsule. It was prepared according to the procedure and evaluated by performing the above test like spredability, stability, appearance etc.

EVALUATION [7-10]

1. ORGANOLEPTIC CHARACTER

The consistency and the colour was checked visually the odour was evaluated manually by smelling the product. The organoleptic character include its color, odor, feel and consistency which were evaluated manually for its physical properties **(Table 2)**.

2. PHYSICAL STABILITY

Observation of physical stability at room temperature by observing organoleptic during storage. This formulation was

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performed to see the stability on formulations at low and high temperature of prepared peel off mask. Six cycle between refrigerator temperature (4°C) and accelerated temperature (40°C) with storage at each temperature for not less than 24 hours performed. The formulation was found to be stable at these temperatures were subjected to Freeze thaw stress test found stable **(Table 3)**.

3. IRRITATION TEST

This parameter checked with patch test. Irritated skin at the patch site may indicate an allergy

Mark an area (1sq.cm) on the left-hand dorsal surface. Definite quantities of prepared peel of mask were applied to the specified area and time was noted. Irritancy was checked if any for regular intervals up to 24 hrs and reported **(Table 4)**.

4. PEELING TIME

The peel gel was applied on the skin surface uniformly. The peel was allowed to dry. After 15 min the peel was removed from the skin surface. It was observed that the peel was removed easily without breaking **(Table 5)**.

5. HOMOGENISITY TEST-

Test Homogeneity testing is investigated by applying a peel-off mask to a glass object or transparent material, then observing the composition of coarse or inhomogeneous particles and recording them. The preparation must show a homogeneous order and should not show any coarse grains **(Table 6)**.

6. SPREDABILITY TEST -

The spreading capacity of peel of mask formulation was measured 48 hr after preparation by measuring the spreading diameter of 1 gm of the gel between two 20×20cm glass plate after 1 min. The mass of the upper plate was standardized at 125g **(Table 7)**.

The following equation was used for the purpose:

$$S=m*l/t$$

Where,

S = the spredability of the mask formulation

m = the weight (g) tied on the upper plate

l =the length of the glass plates

t = the time taken (second)

7. P^H MEASUREMENT

The p^H value of topical peel off mask was determined by using digital p^H meter. One gram of gel was dissolved in 100 ml distilled water and stored for two hours. The measurements of p^H of the formulation were done in triplicate and average values were calculated **(Table 8)**.

Table 2: Organoleptic Properties of the Peel of Mask

PARAMETER	OBESERVATION
Colour	Yellowish Green
Odour	Pleasant
Consistency	Smooth
Nature	Semisolid

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Table 3: Stability Study of the Peel of Mask

Stability	Preparation		
	1 gm	2gm	2.5gm
1day	Stable	Stable	Stable
1week	Stable	Stable	Stable
2week	Stable	Stable	Stable

Table 4: Results of Irritation Tests

Formulation	Observation		
1%	Non irritant	Non irritant	Non irritant
2%	Non irritant	Non irritant	Non irritant
2.5%	Non irritant	Non irritant	Non irritant

Table 5: Results of Peeling Time Study

Formulation	Time
1%	12minute,10 second
2%	14 minute, 37 second
2.5%	15 minute,25 second

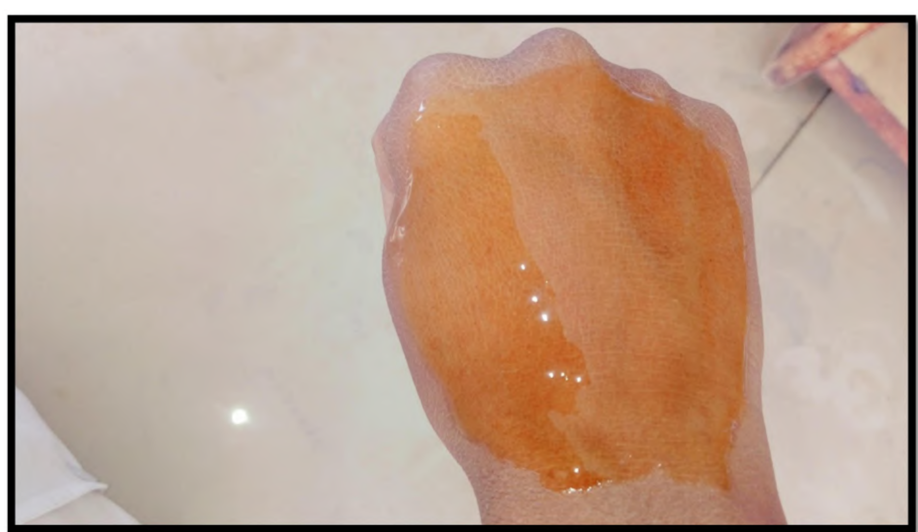

Figure 2: Applied peeling property

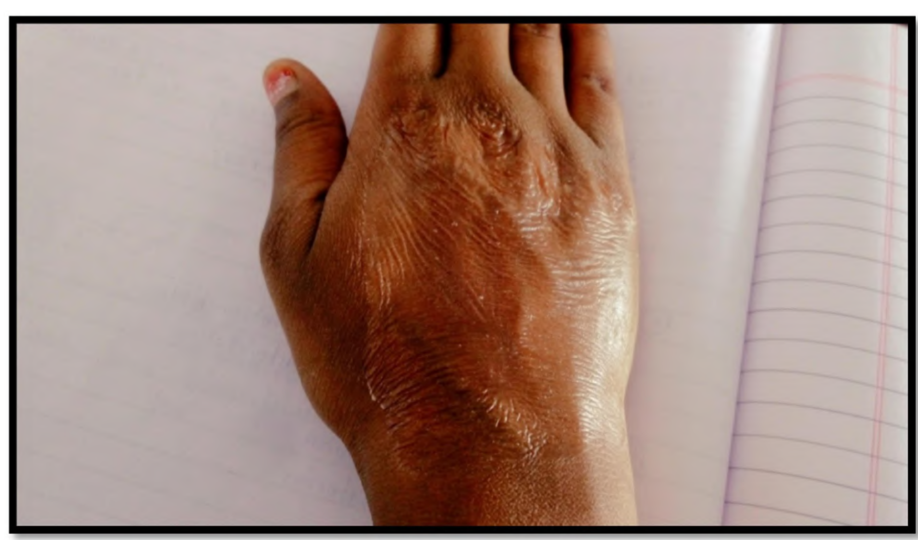

Figure 3: Dried peeling property

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Table 6: Homogeneity of the Formulation

Formulation	Observation		
1%	Gel	Gel	Gel
2%	Gel	Gel	Gel
2.5%	Gel	Gel	Gel

Table 7: Spreadability Study of the Formulation

Formulation	Observation		
1%	0.900 cm^2	1.8 cm^2	2.3 cm^2
2%	0.92 cm^2	1.5 cm^2	2.2 cm^2
2.5%	0.7 cm^2	1.57 cm^2	2.0 cm^2

Table 8: pH evaluation of the Peel of Mask

Formulation	P^H Observation		
	1Day	1week	2week
1%	5.3	6.0	6.8
2%	5.5	6.2	7.2
2.5%	5.1	6.5	7.5

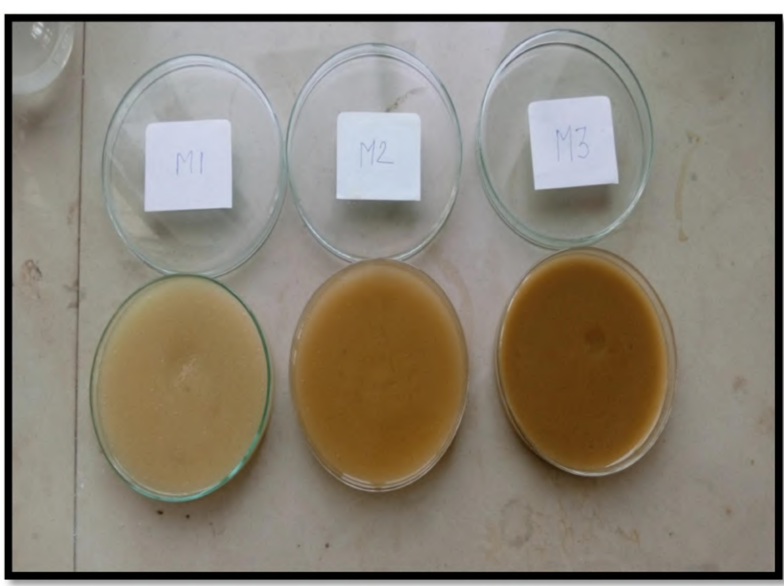

Figure 4: Formulation of Peel of mask of aloe vera and vitamin E

Figure 5: Packing and Labeling of Final Product

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CONCLUSION

The Aloe vera and vitamin E peel off mask was found to successful with good results. The peel off mask showed a good spredability. The formulation showed a good peel off property on human skin without causing skin irritation. The formulation showed good stability results and was found to be stable till room temperature.

The entire required ingredient to prepare peel of mask Aloe vera powder and vitamin E from the aloe Vera powder extract and vitamin E capsule. It was prepared according to the procedure and evaluated by performing the above test like spredability, stability, appearance etc.

Aloe vera and peel of mask was prepared and evaluated by doing various test. evaluation tests were carried out and confirmed the product sensitivity and appearance. Aloe vera and Vitamin E peel of mask successfully passed all tests such as Organoleptic characters, Physical stability, Irritation Test, Peeling Time, Homogenisity Test, Spredability Test and PH Measurement.

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Building a Self-Driving Autonomous Car Model Using the Raspberry Pi Processor and Computer Vision Methods

Kajal Pawar[1*], Prathmesh Biramane[2], Pramila kamble[3,] Omkar Bhosale[4], Prof. Puranik V.V.[5]
[1-4] UG Student, [5]Professor, Department of Electrical Engineering, Yashoda Technical Campus, Satara, Maharashtra, India

[*]Corresponding Author: kajalpawar1701@gmail.com

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ABSTRACT

The development of autonomous self-driving vehicles has attracted a lot of attention in the transportation industry recently. The building of a self-driving autonomous automobile model employing a 4B 8GB RAM Raspberry Pi processor and computer vision algorithms is demonstrated in this project. The vehicle has a Raspberry Pi camera in the front for object recognition and an ultrasonic sensor in the back for obstacle recognition. Using image processing methods, the suggested model is capable of detecting objects, lanes, and traffic signals. The vehicle is also capable of making choices depending on the information detected and controlling the vehicle accordingly. Future smart cars that are predicted to be driverless, effective, and crash-avoidant are autonomous vehicles. Automakers have started working in this area to realize the potential and address the issues that are currently present to achieve the desired results. The field of autonomous automation is of interest to researchers and much in this field has been done, of which the present paper has a detailed timeline. This article can help to understand the trends in autonomous vehicle technology for the past, present and future. Since 1920 we see a dramatic change in autonomous vehicle technology when the fest radio-controlled vehicles were designed. In subsequent decades, we see fairly autonomous electric cars powered by embedded circuitry on roads. By the 1960s autonomous vehicles with similar Electronic Guide Systems came into the picture. By the 1980s vision guided SC autonomous vehicles was a major step in technology. Various semi-autonomous features introduced in the modem cars such as Laredo, automated braking and adaptive cruise control are based on such systems. The future of autonomous vehicles is extensive network-aided systems in conjunction with vision-driven features. By the advent of the next decade, most companies will launch fully autonomous cars. The autonomous vehicle future is an ambitious era of safe and comfortable transportation.

Keywords- Circuit, Lane detection, Predictive modelling, Self-driving, Smart discrimination

INTRODUCTION

The development of self-driving autonomous cars has become a viable alternative for improving transportation safety and efficiency. Autonomous vehicles have the potential to eliminate human error and make driving more convenient for individuals. We propose a self-driving autonomous car model employing a 4B 8GB RAM Raspberry Pi processor and computer vision algorithms in this research. The car is programmed to identify traffic signs, lanes, and objects on the road and make appropriate decisions [1].

LITERATURE VIEW

Rasheed Hussain and Shefali Zeadally's [2] article, "Autonomous Cars: Research Results, Issues, and Future Challenges," is available online. The recommended system is an autonomous vehicle prototype with several objectives, such as object detection, path detection, and traffic signal detection using the Raspberry Pi's processor, camera, and ultrasonic sensor for the aforementioned functions. Ultrasonic sensors are used to steer clear of obstacles and detect them. Traffic signals, signs, and walkways are all detected by a camera. Motor drives enable the direction-changing,

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starting, and stopping of motors.[3] A Unified Map-Based Autonomous Driving System for Unknown Environments by Jongwon Choi [4]. Autonomous vehicles have made significant advancements and will be key components of future intelligent transportation networks. These cars must have the ability to independently adhere to traffic laws while avoiding Short Term Traffic Prediction for Enhanced Autonomous and Connected Cars using Edge Computing- Hui-Nien Hung, Shun-Ren Yang, Yu-Ju Su, Yao-Yuan Chang [5]. Future intelligent transportation systems will be greatly aided by the development of self-driving cars, which have advanced significantly. For these cars to be successfully deployed on real roads, they must be able to autonomously travel along collision-free paths while obeying traffic laws. Instead of using prebuilt maps of highways and traffic signals to identify barriers, other cars, traffic signs, and pedestrians, we propose methods and systems that use a single map made using a variety of onboard sensors. The suggested map not only provides details about nearby physical obstructions but also virtual ones like traffic. Based on realistic road data and typical sensor accuracies, simulations are run across driving distances of about 150 km and the navigation system's layout is explored. Positioning errors less than 10 m (standard deviation) in size are seen. The synchronization error between measured and mapped data must be continuously assessed to reach this accuracy. The newly released Navistar GPS-based navigation technology is perfect for completing current, commercial automotive navigation systems. The path planner may effectively discover collision-free paths while adhering to traffic regulations using this map. The proposed algorithms were tested on a commercial vehicle and proven in a variety of scenarios, including the 2012 Hyundai autonomous ground vehicle competition [6].

COMPONENTS

Power Supply

The power supply circuit has two additional pins for attaching a transformer. These pins charge the battery and supply the required DC voltage to the bridge rectifier, which has a PIV (Peak Inverse Voltage) rating of 1000V. To generate a smoother DC waveform, the rectified pulsing DC output is sent to a 1000uF capacitor. This smoothed DC voltage is then sent into the 7805 IC, which produces a stable 5V output, and the 7812 IC, which produces a stable 12V output. In addition, a 470-ohm resistor is included in the circuit to manage the current (Fig. 1)[7,8].

***Figure 1**: Battery for power supply.*

Raspberry pi

The central component employed in the project is a compact single-board computer with dimensions like a credit card. This computer is specifically designed and programmed to perform image recognition tasks, enabling it to analyze and compare images. Once the analysis is complete, the computer executes an algorithm to determine the most appropriate course of action based on the input image. In essence, it leverages its training and processing capabilities to swiftly respond to the given visual information (Fig. 2) [9].

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***Figure 2**: Raspberry Pi microprocessor.*

Motor Drive

The purpose of this circuit is to control the movement of the model by driving two DC motors. The motor driver utilized in this circuit is the L293D, capable of independently operating two DC motors simultaneously. Input signals for controlling the motor driver are received from the GPIO (General Purpose Input/Output) pins, specifically pins 12, 16, 20, and 21. These input signals dictate the desired movement and speed of the motors, allowing for precise control and coordination (Fig. 3) [10].

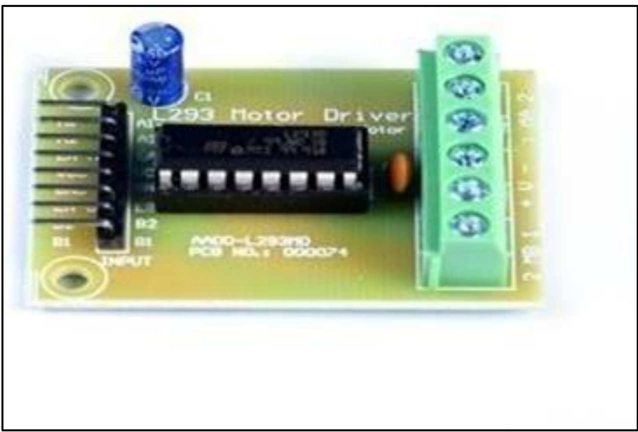

***Figure 3**: Motor drive to control motors.*

Ultrasonic Sensor

It is used to calculate distance. The ultrasonic trig and echo pins are linked to Raspberry Pi GPIO pins 17 and 18, respectively. (Fig. 4) [11].

***Figure 4**: Ultrasonic sensor for sensing.*

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Camera

The camera is linked to the raspberry pi via USB and will capture images from its surroundings and send them to the raspberry pi for processing (Fig. 5).

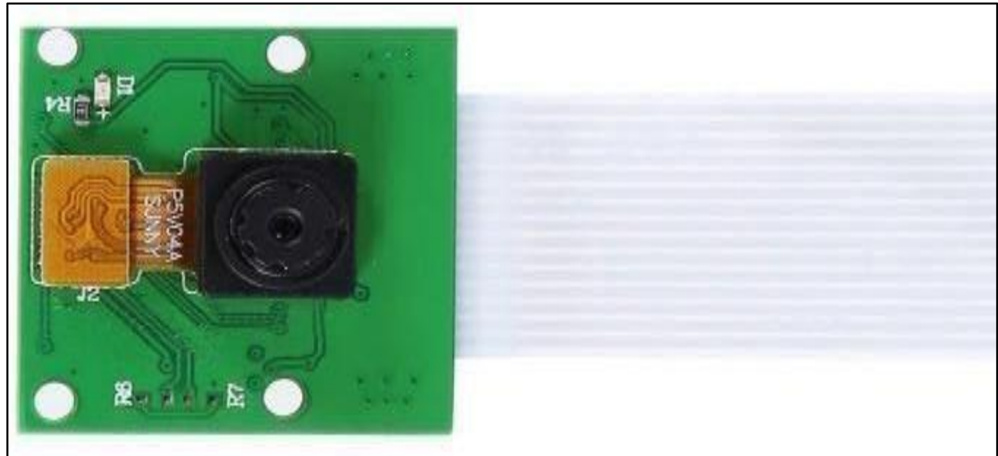

Figure 5: *Camera for detection.*

PROPOSED SYSTEM

The present suggested system employs a pattern-matching technique in which cameras detect a unique pattern that would be printed on the roads. This pattern will be captured by the camera and processed using a Raspberry Pi to command the automobile to travel in the given direction. The camera will also capture surrounding images to determine different obstructions adjacent to it; if the obstacles get too close or are going to collide with the vehicle, the vehicle will stop until the obstacle close to it moves. Special patterns will be deployed alongside the route to detect the type of road ahead. The Pi Camera will be installed on the vehicle. It will be connected to Raspberry Pi 3B+. The camera will capture all the images and send the data to the Raspberry Pi for processing. The Raspberry Pi will be powered by a 5V power supply. We will install the Raspbian Stretch OS. The Genny editor will be used for programming purposes. Python will be used as the programming language. The L293D motor driver will start the dc motors based on the Raspberry Pi instructions [12].

OPERATING METHODOLOGY

Computer Vision

Vision in computers because there was insufficient time to build it properly, a computer vision algorithm was not implemented in the core algorithm of this project. However, there existed a camera with which some testing was conducted, as well as an algorithm written in Python to work with OpenCV. This algorithm developed by a member of the computation team working on this was able to detect the presence of traffic signals and to detect some of them in real-time; however, even though this was possible for a small number of signals, it proved difficult to work with all of them because they were too numerous and would significantly slow down the main programme. It was planned to use a database confined to the signals that the vehicle would encounter, as well as a neural network that would learn the signals and then not need to search for matches, but none of these was eventually implemented. A couple of MATLAB programmes were created for road detecting one for detecting road lines in a normal road and another for detecting asphalt based on textures and/or colour [13-16].

Path Planning

Path planning entails developing a series of states from the car's present state to the next objective state, which does not describe how the car's states evolve. Path planning is typically divided into global and local paths [KAM04]. A global path is generated before the car begins travelling using an offline global map of the environment in global path planning. A local path is built as the car is travelling utilising online local maps of the surroundings, allowing the automobile to deal with moving obstacles.

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Path planning methods can be divided into two categories: graph search-based and interpolating curve based [17].

BLOCK DIAGRAM

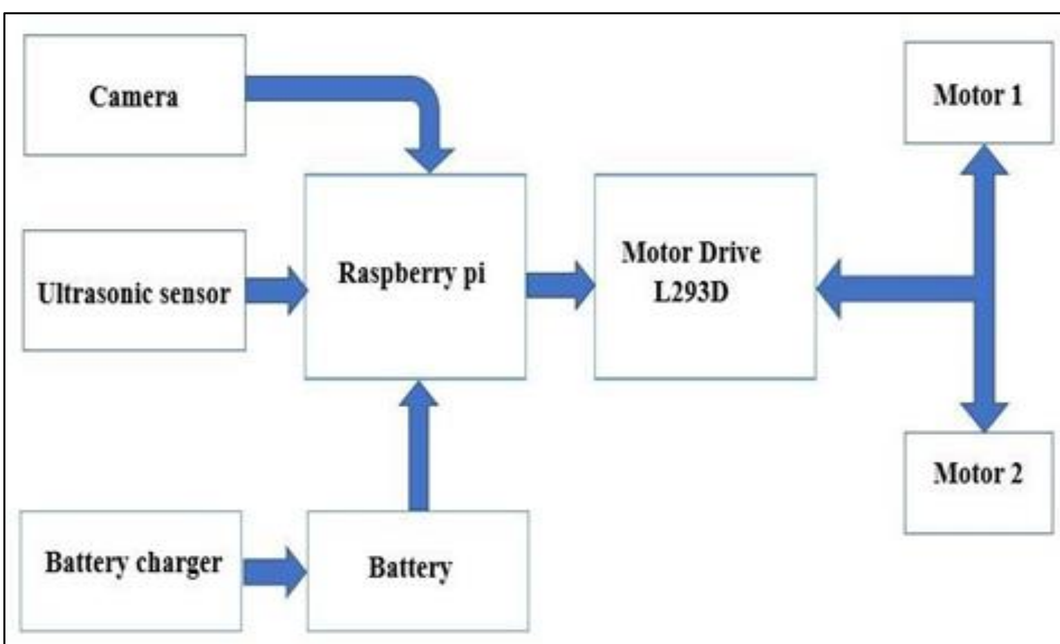

***Figure 6**: Block diagram of construction for the autonomous car.*

The proposed system is a prototype function of an autonomous car with multiple objectives, like object detection, path detection and traffic signal detection. The above functions are implemented with a Raspberry Pi processor, camera and ultrasonic sensor. The ultrasonic sensor is used to detect obstacles and avoid collision. The camera is used to detect paths, traffic signs and signals. The motor drive is used for direction change and start and stop of motors (Fig. 6, 7).

***Figure 7**: Autonomous car with connection.*

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CONCLUSION

A self-driving autonomous car model using a 4B 8GB RAM Raspberry Pi processor and programs for computer vision was presented in this project. The vehicle had an ultrasonic sensor for the back to identify obstacles and a Raspberry Pi camera for the front to detect objects. The suggested model could recognize objects, lanes, and traffic signs along the way and make decisions depending on the information it had picked up. Obstacles in the back of the car could be found and avoided with the help of the back ultrasonic sensor. The outcomes demonstrated that the suggested model was successful in following the path with high accuracy and without requiring human intervention. As technology advances around the world, self-driving cars will become the dominant means of transportation in the future. The legal, ethical, and societal consequences of self-driving cars revolve around concepts such as liability, responsibility, and efficiency. Automobile cars will benefit the economy through increased fuel efficiency, the environment through reduced carbon emissions, society by increased togetherness, and the law through simplified liability systems. These concepts, however, concentrate on two essential features of autonomous vehicles: how they work and how they are secured. As technology advances, security technologies for self-driving cars will develop to combat hackers, improve internal system accuracy, and prevent accidents. When all of these technologies are at their peak, civilization will be one step closer to the ideal of flying vehicles that most of us dreamed of as children.

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Smart EV Charging Station With ON Grid Green Power & Wireless Charging

Basawaraj Hebbale[1], Prasad. A. N[2*], Shinde. N. S[3], Abhijeet. S. S[4], Suraj. D. G[5]
[1]Assistant Professor, [2-5]Under Graduate Student, Department of Electrical Engineering, Yashoda Technical Campus, Satara, Maharashtra, India

[*]Corresponding Author: prasadashoknalawade@gmail.com

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ABSTRACT

Emerging nations like India are deploying electric vehicle (EV) technology and phasing out the use of fossil fuel-powered vehicles as part of their effort to tackle climate change and rising urban pollution. In April 2017, the Indian government declared that all EVs would be sold on the market by 2030. Also followed is the promotion of the FAME (faster adoption and production of electric vehicles) program. The infrastructure for electric charging is a crucial part of the ecosystem for electric mobility. The market for EV charging stations must grow and accept EVs at the same rate. EVs are constrained by their speed and range. The key to successful electric vehicle use is the network of charging stations that are available. Electric vehicles (EVs) will be smoothly incorporated into the transportation system, which is one of the key elements of future smart city planning. The primary energy source for EVs is a charging station, and a city's accessibility to EVs depends on the station's location. They should be placed thoughtfully so that an electric vehicle (EV) can access a charging station within driving distance and travel anywhere in the city once it has been recharged. In this article, we formulate the Electric Vehicle Charging Station Placement Problem, in which we want to reduce the overall construction cost while keeping in mind the drivers' convenience and the charging station coverage requirements. We examine the problem's characteristics, particularly its hardness.

Keywords- Power supply, Powerful vehicle, Renewable energy, Solar energy, Wireless

INTRODUCTION

Due to growing awareness of the benefits of living sustainably, the adoption of electric vehicles as gas-powered vehicles substitutes has grown quickly.

The grid has traditionally been used for charging electric vehicles. However, due to technological advances in solar energy, it is now possible to recharge electric vehicles using solar-powered chargers. These clean solar chargers supply clean electricity to electric vehicles while also benefiting the environment. Furthermore, the availability of these charging stations would inspire people to reassess their transportation preferences and switch to zero-emission vehicles.

As electric vehicles become more affordable each year, investors have begun to invest in charging infrastructure grid supply for widely available automobiles, to design and create a solar-powered charging station, to gather electric vehicle power information, and put the charging station into operation with the capability of utilising solar energy when it is available and switching to grid supply otherwise. A charging station supplied by a traditional grid supply has numerous restrictions and disadvantages, thus we employ solar energy for charging. The switching circuit allows circuits to be switched, and the application of MPPT (maximum power point tracking) allows maximum solar energy to be tracked.

The market for electric cars (EVs) is gradually expanding, to fast recharge the car; the current conductive charging method requires high-power charging equipment or charging stations.

The different EV models' incompatible plug receptacles add to the inconvenience. As with wireless charging systems, several EV models can share the same charging

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infrastructure.

85 million autonomous vehicles are projected to be in operation by 2035, which necessitates the development of wireless charging [1, 2]. WPT, as opposed to wires, makes the system simpler, automatic, secure, inexpensive, and more efficient. Wireless charger solutions, for example, enable automated charging while EVs are temporarily parked in parking lots.

SYSTEM DESCRIPTION

In this approach, the car is charged via the plug-in method. This has various shortcomings, such as the size of the battery. The battery's large size makes acceleration lessen. The battery is the major obstacle to bringing electric automobiles into the affordable price range. Smaller batteries would be much cheaper, thus wireless charging was used. Therefore, wireless charging is more user-friendly and has a faster charging rate because there is no need to plug a cable into the automobile [3].

METHODOLOGY

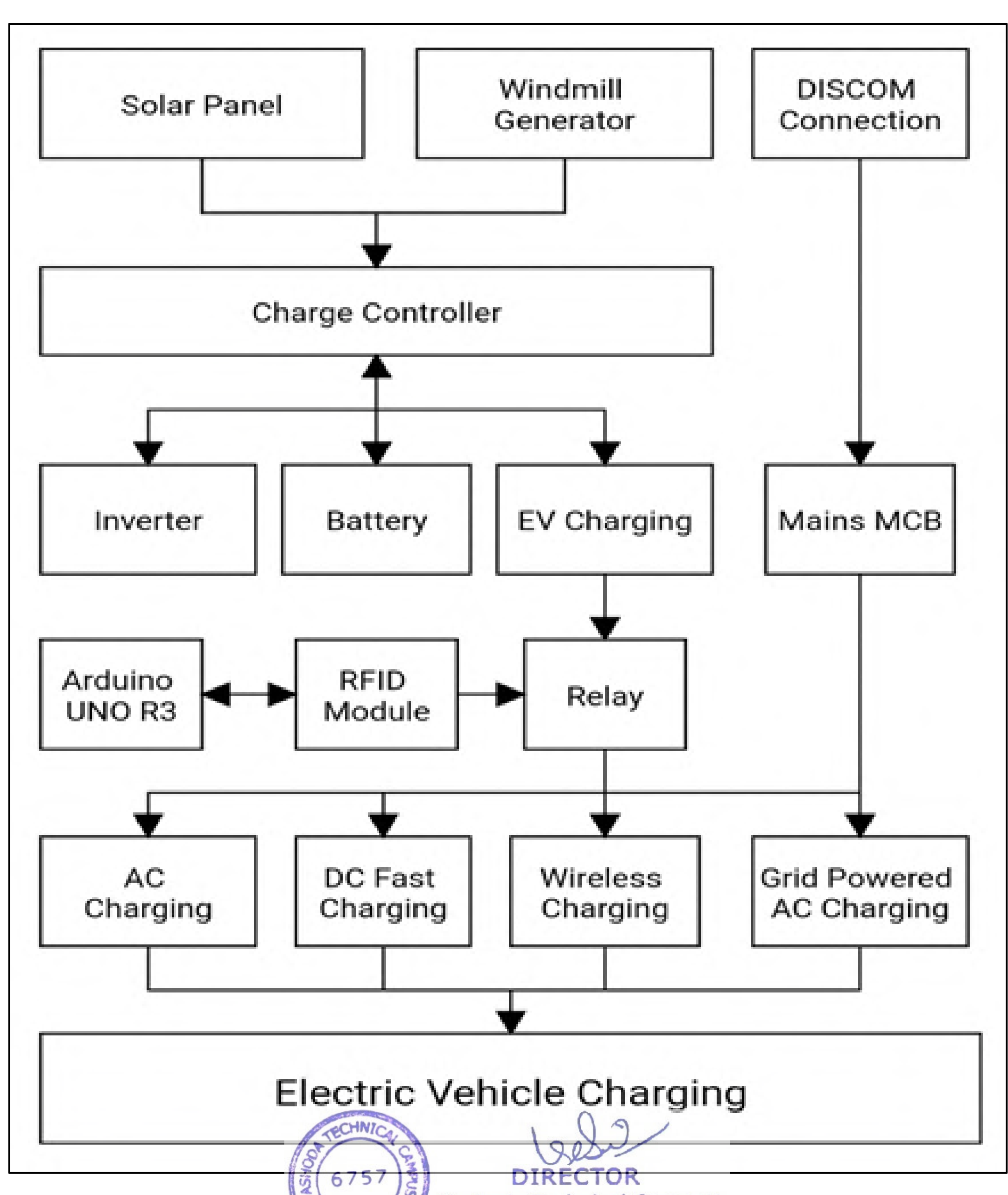

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Figure 1: Block diagram of charging methods.

An EV charging station must include an inverter with a grid interface, an RCL filter, a transformer, a DC bus feed, and battery chargers. The two forms of renewable energy sources utilized in this project are solar energy and wind energy. Firstly, the components that convert natural phenomena into electrical energy, such as solar PV cells and wind turbines. Both solar PV cells and wind turbines frequently use Buck-Boost converters, which ensure that low voltage is increased into necessary high voltage so that the battery may be charged. Because this circuit only converts AC to DC in one direction, a rectifier with C smoothing is only used at wind energy sources (Fig. 1, 2).

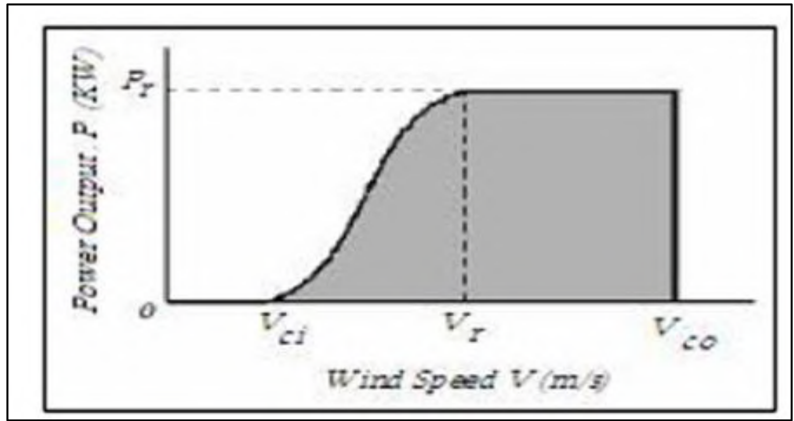

Figure 2: Waveform of wind energy output.

WORKING

Let us say that the transmitter has L1 turns, I1 current, and a magnetic field. A little amount of magnetic flux may flow through the receiver when the transmitter and receiver are close together [4, 5].

If the transformer has a primary and secondary side, charging without a WEVCS connection has a transmitter side and a reception side. A wireless charger for an electric vehicle's transmitter and receiver windings are equivalent to the primary (coil) and secondary windings of a transformer. However, wireless charging of electric vehicles shifts the alternating current (AC) parameters from low frequency 50Hz to high frequency, which does not occur in the transformer. Before the alternating magnetic field is formed, the transmitter coil gets high-frequency alternating current power. This field then stimulates the receiver winding, causing a voltage to appear on it. This voltage is utilized to charge the battery in the car [6].

The frequency of resonance between the transmitter and receiver must be maintained for wireless charging to function properly. Compensatory networks are introduced on both sides to keep the resonance frequency constant (Fig. 3).

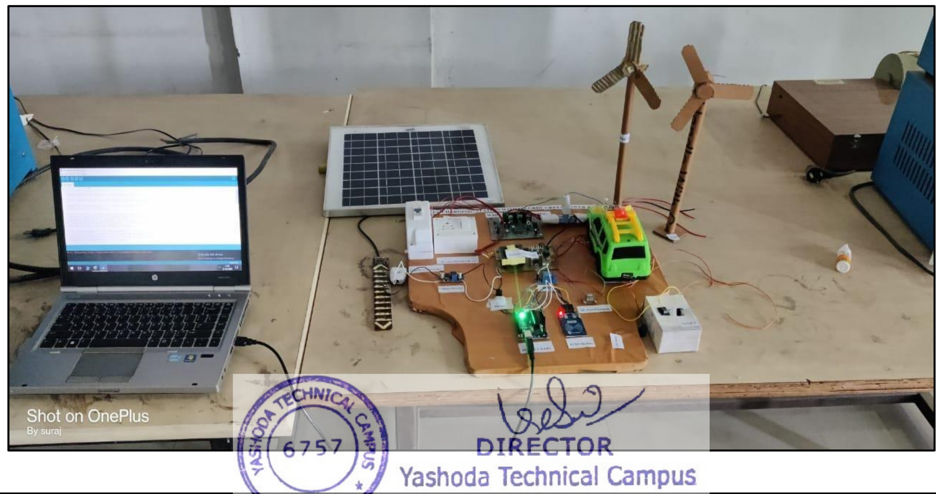

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Figure 3: *Working project module.*

Modes of Charging

Mode 1 is the cheapest and simplest method for charging at home, but it is also the slowest. Electric cars (EVs) include a built-in battery charger as well as connections that allow owners to plug their vehicles into a garage outlet. EVs can be charged using a typical 230 V household socket, although the available current is only 16 A, according to mode 1. Although charging periods vary widely from vehicle to vehicle, a tiny electric car's battery typically needs 7 to 15 hours to recharge to its full capacity. The EV is connected to the outlet using conventional industrial plugs and sockets in This method of charging is not allowed in the USA since it

Mode 2: This charging method uses single-phase or three-phase 230 V or 440 V alternating current mains with a maximum current of 32 A. Similar to Mode 1, no special plugs or sockets are required to connect EVs to garage wall boxes or charging stations located in public spaces, such as a restaurant, mall, city park, or even a place of employment. The circuitry required to carry out safety functions, such as i) confirming that the vehicle is connected properly, ii) routinely checking the continuity of the protective earth conductor, iii) energizing the system, and iv) de-energizing the system, is present in charging boxes and/or charging stations as well as the on-board battery charger. In general, Mode 2 charging is referred to as "opportunity charging" because it is frequently used by vehicles [7].

Mode 2: charging takes around 3-5 hours for a full charge of a compact car.

Mode 3: It is generally powered by a three-phase 440 V alternating current and employs specialized plugs and sockets to supply up to 63 kW to the onboard battery charger. In addition to Mode 2's security features, charging stations and onboard battery chargers adhere to the proper protocols for coordinating their operations. Because of the higher available power, a mode 3 charge can fully charge a small vehicle in under an hour. Electric buses, for example, require mode 3 charging, which is available in public and commercial areas, airports, and transportation hubs.

Mode 4: The charging station's rectifier converts alternating current mains power into direct current voltage. The EV receives up to 400 A from a special connector on an off-board battery charger. The Japanese standard "CHAdeMO" is the most extensively used mode 4 charging option. With a power output of up to 50 kW, it can charge a tiny car in under 30 minutes.

WPTS Technology for Electric Vehicle

In 1996 and 1997, General Motors released two EVs, the EV1 and the Chevrolet S-10 EV, which employed the Magne Charger, also known as J1773, which utilized the principle of inductive power transfer. In place of a plug, a "pad" carrying the primary coil is put into the EV's slot. The secondary coil is housed in the slot, and when combined with the pad, a Transformer for wireless power transfer (WPT) is produced. These pads did not perform as well as they could have. However, these pads required manual insertion into the EV, making them equally as problematic as conventional plugs.

However, with growing interest in e-mobility, there is a lot of research interest in making EVs a good option for future transportation.

WPT charging technology can positively affect people's attitudes towards EVs. It is expensive, has a limited driving range, and has a lengthy charging process. However, with the development of WPT technology for charge replenishment, an EV scan has become a desired option. WPT charging provides the advantage of being able to automate, simplify, and secure the charging process for users. The broad adoption of WPT charging infrastructure may also help reduce the size of the battery pack, increasing the efficiency of EVs. Traditional inductive chargers are incapable of handling all of this, necessitating WPT charging via vast air gaps and minimal human touch. GM, Qualcomm Halo, Delphi, and others are among the largest manufacturers [8].

SIMULATION

Dependence on renewable energy sources would be the key response to the crisis. As a result, this project includes a solar-powered grid-connected system with electric auto rickshaw charging capacity. To discuss the influence of solar energy, the study used correct result analysis.

Simulation of Solar System

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A 6 kW solar system is selected for the performance investigation of a Solar Powered Grid Connected Charging Station. The infrastructure will be put in place at Chinnakada, Kollam, Kerala, India to make it easier for electric auto rickshaw drivers to charge their vehicles. Solar panels are used to offset the energy given to the load from the grid. The 6kW system was chosen based on the load profile of the available electric vehicles in the area. To meet future demand, the system's capacity can be increased. A simplified schematic diagram represents it (Fig. 4).

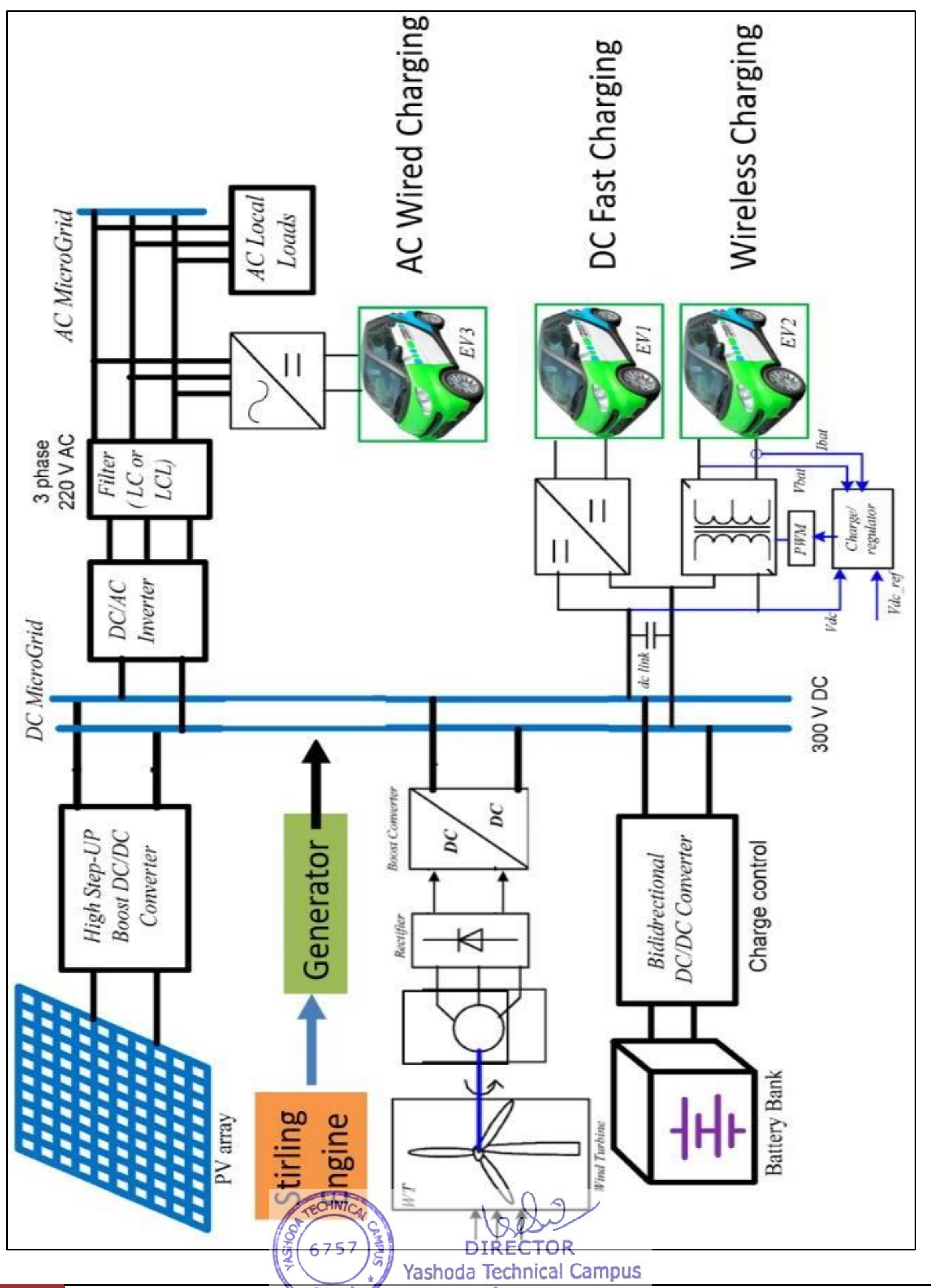

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Figure 4: *Block diagram of inputs and outputs of charging.*

A PV array can produce the necessary demand. The system is connected to the grid, so any additional power requirements are satisfied by utilising the grid's supply of electricity. Three subsystems comprise the overall schema.

RESULT AND DISCUSSION

Inverter output signal while the battery is being charged and discharged then Charging and draining at the battery end A 440V AC supply is used as the device's input, and a smoothing capacitor linked in parallel with the circuit allows for variable output DC voltage [9, 10].

Observation

By raising the capacitor value, the output voltage is smoothed. The (Is) n = n=1, 3, 5 (4IO/n) cos(n/2) sin(nt-n/2) supply the output waveform's output voltage and current harmonics. As a result, decreases the output harmonics and becomes inversely proportional to the capacitor value.
However, there are two important factors to take into account while selecting a smoothing capacitor. The operating voltage must be larger than the no-load output value to function the rectifier, and the capacitance value, which determines how much ripple, is superimposed on top of the DC voltage. When the capacitance is too low, as with a 1 microfarad capacitor, it has no impact on the waveform of the output. The output voltage will be almost as smooth as pure DC if the smoothing capacitor is large enough (parallel capacitors can be utilized) and the load current is not too high.

Inverter Output for Charging

The output voltage, output current, and source current of the inverter are determined using Simulink while maintaining the value of the LC elements. The result therefore acquired is filtered with an LC filter to get rid of the harmonics. Under standard testing conditions, the specifications are - Hz or fifty hertz.

The output voltage and current frequency can be changed by altering the capacitor and inductor values. We can see from the Simulink findings above that the output voltage frequency rises while the output current falls under the influence of harmonics controlled by the LC filter. It is known that the increase in the capacitor value decreases the output waveform harmonics and hence the LC filter alongside the inverted produces a close-by ideal AC input for the charging station hub. The SCR firing angle (α) is changed to meet desired output load requirements for EV charging (Fig. 5, 6).

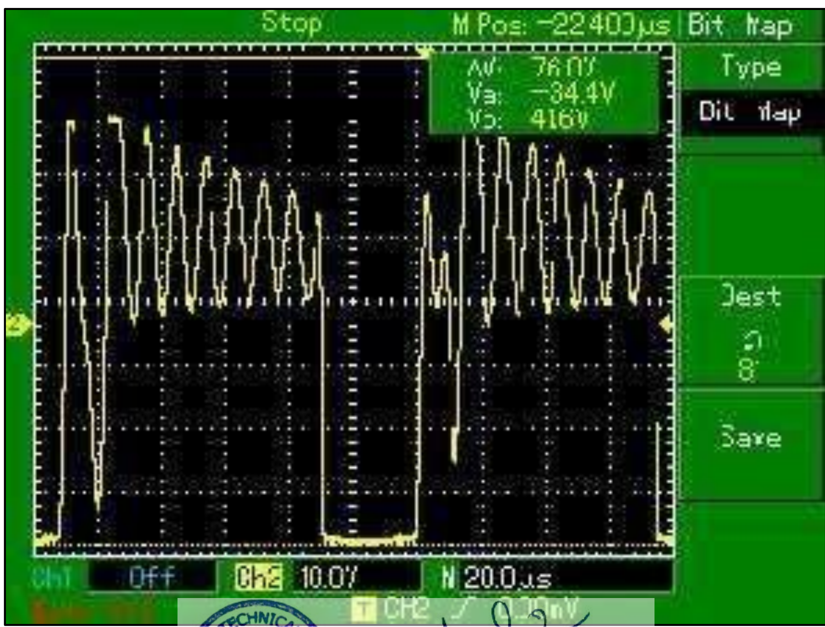

Figure 5: *DSO output voltage of primary coil without compensation and its parameters.*

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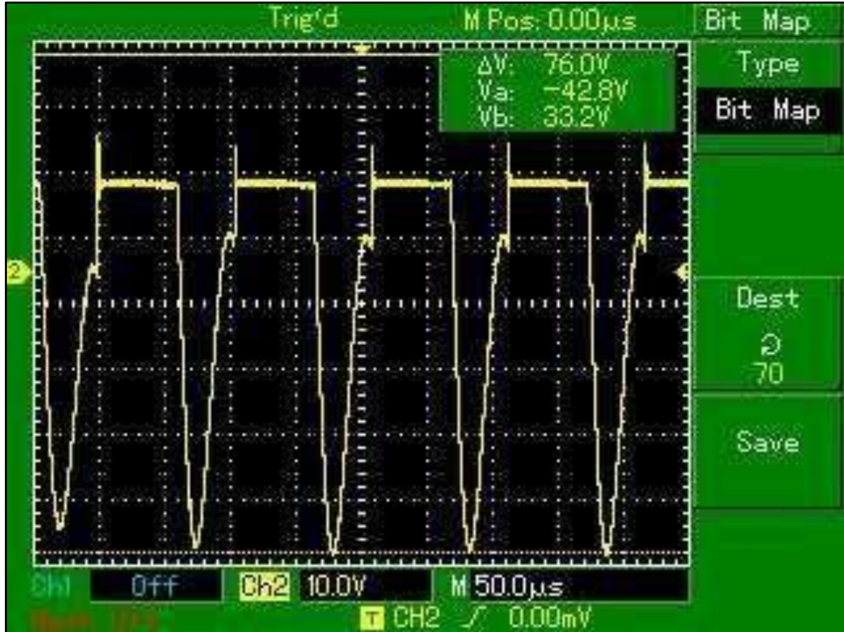

Figure 6: *DSO output voltage of secondary coil voltage without compensation and its parameters.*

Final Output

There are three kinds of standard EV charging stations. AC is used for level 1 and level 2 charging while DC is used for level 3 fast charging, which is primarily used in European nations (Table 1).

Table 1: *Charging station ratings.*

Parameters	Level 1	Level 2	Fast Charging
Voltage	120 V	220-240 V	200-450 V
Maximum Current	16 A	80 A	200 A
Current Type	AC	AC	DC
Power	1.4 KW	7.2 KW	50 KW
Maximum Output	1.9 KW	19 KW	150 KW
Charging Time	12 Hours	3 Hours	20 Minutes

CONCLUSION

Currently, the necessity to lessen emissions while conserving energy is the main factor driving domestic and international adoption of electric vehicles. Using renewable energy sources can save energy while reducing emissions. The development of renewable energy can considerably increase energy conservation.

- Solar-wind hybrid power plants, when combined with electric vehicles, will help to cut energy consumption and greenhouse gas emissions in the transportation sector.
- Fuel-based transportation has significantly increased pollution problems. To give a more beneficial option, the ICPT principle is employed to address the significant drawbacks associated with charging electric vehicles in the current situation. Thus, this project offers a template that may be applied to wireless charging at various parking lots.

Voltage changes as coil spacing changes. The model is scaled at a ratio of 100:1 with power levels up to 15 watts, and significant power is transmitted up to 35mm utilizing PP topology, which will increase efficiency when scaled in real-time.

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UTILIZATION OF M25 GRADE CONCRETE BY PARTIAL REPLACEMENT OF CUPOLA SLAG FOR COARSE AGGREGATE

Yewale Sourabh Sahebrao*1, Raut Prashant Balasaheb*2, Chavan Vishwas Raju*3, Phalke Kishor Haibatrao*4, Vanjari Dnyaneshwari Lalaso*5, Shah Ajinkya S*6

*1,2,3,4,5UG Student, Department Of Civil Engineering, Yashoda Technical Campus,

Satara – 415011, Maharashtra, India.

*6Assistant Professor, Department Of Civil Engineering, Yashoda Technical Campus,

Satara – 415011, Maharashtra, India.

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ABSTRACT

An abstract is a summary of entire paper should be written in Times new roman with font size- 10. The abstract The Indian steel industry has been growing rapidly over past few years, driven by increased demand from various sectors including construction, infrastructure and railways cupola slag is by product of steel industry and it is generally disposed into the landfill without treating properly. In this experimental study we are partially replacing the coarse aggregates by using cupola slag in (0%, 5%, 10%, 15%, 20%, 25%, and 30%) with water cement ratio of 0.45 in M25 grade concert. We tested compressive strength after 7 & 28 days of curing. It is observed that maximum compressive strength attain was 40.22 N/MM² at 25% for 28 days of curing at the same time it is observed that the compressive strength reduced up to 26.07 N/MM² at 30%. Also we observed that the minimum compressive strength attain was 25.70N/MM² at 5% .overall, the study concludes that the use of cupola slag as a partial replacement of natural coarse aggregates in m25 grade concrete can be a viable option provided that the replacement levels are kept below 30% and the mix design parameters are carefully optimised to maintain the desired strength and durability properties of the concrete.

Keywords: Concrete, Cupola Slag, Compressive Strength, Split Tensile Strength, M25 Grade Concrete.

I. INTRODUCTION

The construction industry in India has undergone significant growth over past few decades. The sector has been major contributor to the country's economic development and infrastructure development. In recent year the industry has witnessed significant modernization and technology upgrades, using conventional materials for the development of concrete to provide better strength durability and cost effectiveness is one of the upgrade construction industry is adopting now days.

[1]For the development of various infrastructure the main constituent is concrete with a typical density of 2400kg/M³. This means for the given volume concrete will weigh more than any other construction material concrete is a composite material that contains of cement, water, and aggregates in the unit volume of concrete aggregates hold up to 50-60% of volume. The main source of aggregates in construction industry is from mining the mountains with the help of stone crusher.

[2]Which can have significant envormental impact, both in terms of immediate effects as well as the long term effect on the ecosystem. Extraction of aggregates from mountains can cause soil erosion, deforestation & habitat destruction which can harm the biodiversity of the area.

[3]The construction industry in India is expected to grow at a compound annual growth rate (CAGR) of 15.7% between years 2021-2026. As the construction industry grows the demand for the aggregates produced by the stone crusher industry is likely to increase as well. Using alternative source of aggregates, such as cupola slag can help reduce the dependence on natural source of aggregates and helps mitigation the envormental impact of the construction industry

[4]Cupola slag is a by- product of the steel industry which is generated during the melting of iron and steel in a cupola furnace. The chemical and physical properties of the slag can vary depending on the type of metal being melted, he temperature of the furnace. Also using cupola slag in concrete will help in reducing the self-weight of the structure as well as the overall cost of the structure.

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II. METHODOLOGY

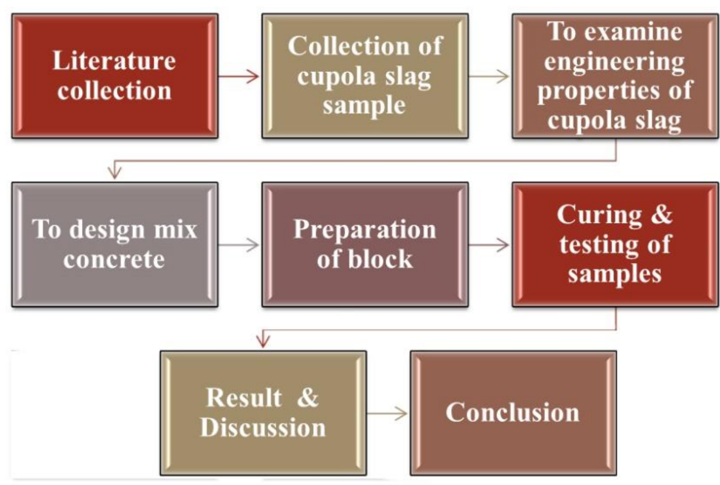

Figure 1: Methodology chart

III. OBJECTIVES

- To study engineering properties of cupola slag.
- To reduce waste generated from steel industries.
- To prepare concrete blocks by using slag as partial replacement for coarse aggregates.
- To compare with normal aggregat econcrete and cupola aggregate concrete.

IV. MODELING AND ANALYSIS

1 CEMENT – We used OPC cement of grade 53. The 53 Grade OPC has a higher strength concrete. As per BIS requirements the minimum 28 days compressive strength of 53 Grade OPC should not be less than 53Mpa.

2 FINE AGGREGATE – We used 4.75mm of fine aggregates in our design mix as shown in following image. Fine aggregates are an essential component of concrete because they help in filling the voids between the coarse aggregates, here are some reasons why we use fine aggregates in concrete.

3 COARSE AGGREGATE – We used 20mm sized coarse aggregate in our design mix the main functions of coarse aggregates in concrete are to provide strength and durability to the concrete. They help to distribute the loads evenly across the concrete structure and provide resistance good compressive strength to the concrete.

4 CUPOLA SLAG - Cupola slag is a material generated during the manufacturing process of steel, and it can used as partial replacement of the coarse aggregates, cement, as well as fine aggregates. In this experimental study we are replacing the coarse aggregates with cupola slag. We collected the sample of cupola slag from Jotirling founders, shiroli, Kolhapur. And graded them into the aggregates in size between 10mm – 20mm coarse aggregates.

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Figure 2: Cupola Slag

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Cupola slag can possess physical properties similar to traditional coarse aggregates, such as suitable particles size distribution and sufficient strength while specific properties may vary depending on the characteristics of the slag obtained from the steel industry careful, grading & selection can ensure that cupola slag adequately perform as replacement, material.

V. RESULTS AND DISCUSSION

1. COMPRESSIVE STRENGTH :

The compressive test on both conventional concrete and cupola slag concrete is carried out in accordance with IS 516- 1999 standards. The test is conducted on concrete specimens of size 150mm x 150mm x 150mm. The specimen is placed at the centre of the compressive testing machine and the load is applied gradually till the specimen fails.

Figure 3: Compressive Strength testing machine.

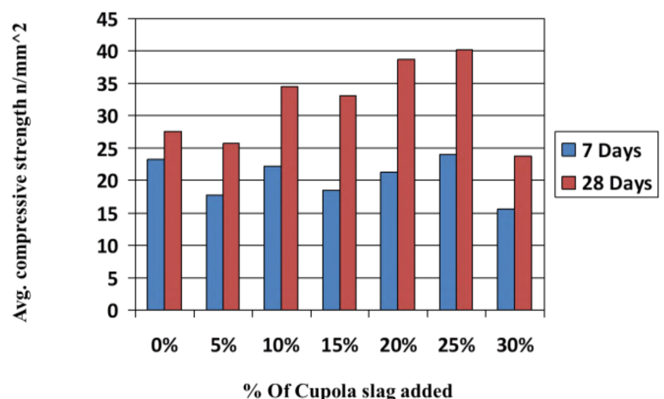

Figure 4 : Variation of compressive strength with variation % of cupola slag

Table 1: compressive strength of blocks after 7 & 28 days of curing

Sr.No.	% of Cupola	Avg. comp. strength 7 days curing N/MM²	Avg. comp. strength 28 days curing N/MM²
1	0%	23.33	31.7
2	5%	17.70	25.70
3	10%	22.15	34.5
4	15%	18.55	33.11
5	20%	21.33	38.14
6	25%	24.00	40.22
7	30%	15.6	26.07

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It can be observed from the results that the compressive strength of the concrete increased by use of cupola slag up to 25% as a partly replacement to coarse aggregate in all concrete mixes & it will be observed that decreases on further increase in cupola slag (30%) replacement in concrete samples. The above result are plotted graphically for curing period of 7 and 28 days.

2. SPLIT TENSILE STRENGTH

The test is conducted on concrete specimens of size 150 x 300mm.The cylindrical specimen is placed at the centre of the testing machine and the load is applied gradually till the specimen fails.

Figure 5: Casting of cylinders for spilt tensile test

Figure 6: Split Tensile Strength

Table 2: Split tensile strength of blocks after 7 & 28 days of curing

Sr. No.	% of Cupola	Avg. comp. strength 7 days curing N/MM²	Avg. comp. strength 28 days curing N/MM²
1	0%	2.47	3.67
2	25%	2.75	3.97

Split tensile strenth of concrete specimen after 7 & 28 days of curing

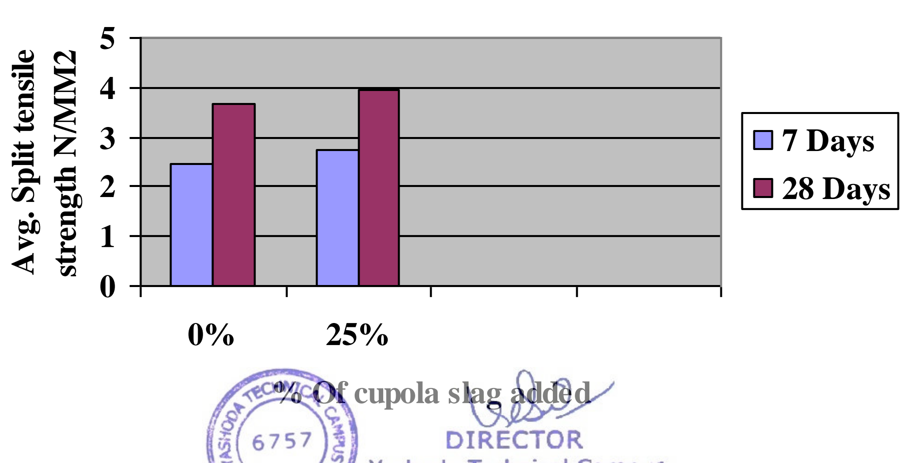

Figure 7: Variation of split tensile strength with variation of % cupola slag

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VI. CONCLUSION

Following are the conclusion of the experimental study:

- The maximum strength achieved by the blocks after 28 days of curing with 25% inclusion of cupola slag is 40.22 N/mm2.
- The utilization of cupola slag, characterized by its low specific gravity and light weight nature contribution to a reduction in the self-weight of the concrete member.
- Cupola slag is widely available at minimal or no cost rendering it an economically viable option for significant construction projects by substituting 25% of the coarse aggregates with cupola slag expenses can be minimised resulting in enhanced cost effectiveness.

VII. FUTURE SCOPE

- Use of cupola slag (25%) replacement percentage for enhancing mechanical properties.
- By optimizing cupola slag in the concrete as partial replacement it will help to reduce waste generated from steel industries.
- Long-term durability studies to assess the performance of cupola slag concrete over time.
- Implementation cupola slag aggregate in concrete is practically possible in real-world construction projects.
- Implementing cupola slag aggregate reduces global-warming and also gives strength.
- Economic feasibility analysis to evaluate the cost-effectiveness and viability of cupola slag concrete.

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PERFORMANCE EVALUATION OF SLUDGE BRICK WITH CONVENTIONAL BRICK

Mr. Sohel M. Shaikh*1, Mr. Huzefa F. Tamboli*2, Mr. Akshay U. Sawant*3, Mr. Rohit S. Kamble*4, Mr. Rohit S. More*5, Mr. Saddam S. Kotwal*6, Mr. P.G. Borate*7

*1,2,3,4,5,6Students, Department Of Civil Engineering, YSPM's Yashoda Technical Campus, Satara, India.

*7HOD, Department Of Civil Engineering, YSPM's Yashoda Technical Campus, Satara, India.

ABSTRACT

Waste is the product of the unwanted material that is from manufacturing Process from industry, from House, or other industries such as agricultural and chemical etc. Waste is harmfull for the environment. It produces many types of disease, infection and problem's to every living being so. The sludge disposal has major concern for human and animal and for every living being The sludge from the water treatment plant has problems of disposal.Therefore, these study is useful for stulge disposal in proper manner with ecofiendly use. So these study we utilize those studge waste (SW) produced from the water treatment plant as using as brick making material. For sludge brick the different proportion and material should use with sludge. Then tests is conducted like water absorption and compressive strength. As the conclustion the 20% sludge is usefull or acceptable to produce good quality brick from these the water treatment plant sludge is suitable ingredient or material for manufacturing of bricks with different proportion, material,mix and design.

Keywords: Sludge Waste, Sludge Brick, Compressive Strength, Water Treatment Plant Sludge, Proportionate Mix.

I. INTRODUCTION

Growth in industrialization and fast growing urbanization is causing major environmental problems. From these major concern is safe and sound disposal of solid sludge waste. There is a strong demand for reuse and effective disposal methods for sludge due to its increasing daily amount of generated sludge by the waste water treatment plants. Sanitary landfills are commonly used for disposal or sludge from sewage,rapid urbanization has made it difficult to find suitable landfill sites or land for disposal. Therefore, Sugar industries, paper pulp and Textile industry are three major agriculture-based industries in India which produce large quantity of solid,semi solid and liquid wastes after consuming greater amount of quality and fresh water. Textile mills are one of the oldest and big sectors in India. Every year textile exports generates large amount of revenues for Indian economy. The proposed method for the manufacture of energy efficient bricks using the sludge from textile industry, thus suggests a means for the waste disposal also. For the past thousands of years, water treatment plant sludge is almost similiar to bricks property. Its chemical composition is also same. Therefore these study shows that sludge is efficiency used as a replacement for brick clay. Throughout It is mainly focused on charactesristics present in brick and its influence on modyfing and thinking of the chemical -physical properties, throughout the concentrations, in water treatment plant sludge the following chemical presents such as Cu, Zn, Cr,Cd and Pb. The natural resources are used for manufacturing of sludge bricks, and as an alternative to conventional or cement bricks which helps in conservation of naturally resources and improves the environment.

1.1 Liquid sludge

The effluent coming from the industries are treated by flocculation process, during this treatment the sludge obtained is called as liquid sludge.

1.2 Semi solid sludge

This is the second form of sludge, obtained by dewatering of liquid sludge by passing through centrifuges at 1500 rpm, because of this high revolution the sludge gets dewatered and comes out as wet cake. This wet cake is called wet sludge.

1.3 Dry sludge

The sludge from the centrifuges is dried by spreading it over a large area, i.e., on the sludge drying beds in the presence of sunlight. Thus, wet sludge is converted into dry sludge in a period of 40 days. All the three forms of

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sludge which has been discussed above are being used as composite material and influence of each on compressive strength and other parameters have been discussed in subsequent articles.

II. OBJECTIVES

2.1 To check Partial replacement of soil by dry sludge as one of the ingredients in brick manufacturing process.

2.2 To reduce in cost of brick and reduction in impact on environment.

2.3 To find if the compressive strength of bricks made using sludge is compatible with conventional brick.

2.4 To examine the effect of dry sludge in brick properties.

III. METHODOLOGY

3.1 Sludge Collection: The collection of sludge can be done from water treatment plant so we got the liquid type sludge we take that sludge in bucket and then put it on the slab for drying of sludge. The waste sludge which is left over drying is allowed to dry for 10days to 14days.The drying process is completely natural.

3.2 Sludge Grinding: After the drying of sludge available water in sludge is evaporated in atmosphere by the sun heat so we get completely dry sludge but in dry sludge we get some lumps so we want to do sludge grinding for getting powdered sludge so we do sludge grinding process.

3.3 Analysis of properties of material used: Here we analyse the different properties of material we are using in mixing with sludge to get good strength and mixing.

3.4 Addition of Cement, Quarry Dust and Sludge in Various Proportion: After analysis we have to decide the proportion of different material used with sludge. First we decide the set1-20% sludge, 20%cement and Quarry dust-60%, Set2- 30%sludge,10%cement,60%Quarry dust, Set3-20%sludge,30%cement and 50%Quarry dust, Set4- 50%sludge,20%cement and 30%quarry dust. These 4 sets for adding proportion we decide.

3.5 Moulding of bricks: In the moulding of brick the required size and shape is given to the prepared brick. There are two types of moulding by hand or by machines.The specimens used for the test includes cubes of (23x10x7)cm for compressive test,The test is conducted for 7 and 14 days.

3.6 Water Curing: After moulding of bricks the brick curing can be done for 7 to 10 days for proper strength and bonding the water curing can be done.

3.7 Air Drying: This process includes the removing of moisture from the surface and coating by using air.It prevents rusting and corrosion which may be caused by redundant moisture.

3.8 Testing: Following First testing done on cement that is fineness test, standard consistency test, The setting time test, specific gravity test is done for cement. Foe quarry dust specific gravity and sieve analysis test carried out and for sludge specific gravity and fineness test. For Sludge brick water absorption and compressive strength should be done.

3.9 Comparing Results: After the testing of brick obtained result should be compared.

IV. TESTING AND EVALUATION

4.1 Testing of Cement

4.1.1 Fineness Test:

The fineness of cement is calculated by passing the cement through 90mm sieve. The dry shrinkage cracks will easily form when the fineness increases.

Fineness of cement = (W1- W2) /W1 X 100

= 6 %

4.1.2 Standard Consistency Test

Co Standard consistency test is the amount of water required to prepare the plastic mix. It is used to find quantity of water for mixing with cement. The consistency of this cement is 33%

Sr. No.	% of water added to cement	Quantity of water added	Initial reading	Final reading
1	25	100	50	40
2	26	4	50	39
3	27	4	50	38

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4	28	4	50	36
5	29	4	50	29
6	30	4	50	20
7	31	4	50	16
8	32	4	50	10
9	33	4	50	6

4.1.3 The Setting Time Test

- Initial setting test
- Final setting test

4.1.4 Specific Gravity Test

With the apparatus density bottle and weighing balance, the following procedure was carried out to determine the specific gravity of cement. 100gm of cement is weighted 990 ml of kerosene is filled in a specific gravity bottle. sample of cement is placed on specific gravity bottle, till the level of kerosene reaches 100ml mark the quality of cement placed in the bottle is calculated. Then the specific gravity of cement is found out by using he following reaction specific gravity of cement =weight of cement (volume of 10ml by weight of kerosene of equal volume of cement.

Specific gravity of cement = 3.19

4.2 Testing of Sludge

4.2.1 Specific Gravity Test

The same procedure was followed to determine the specific gravity of sludge. Instead of cement we used sludge in that procedure.

Specific gravity of sludge = W5X (W3- W1)/ (W5+W3- W4) X (W2- W1)

by calculating using the formula we got specific gravity of sludge as 2.90

4.2.2 Fineness Test

The fineness test was done for the dry sample of sludge. 100gms of sludge was sieved horizontally for about 10 to 15 min and then residues in each sieve was weighed. From that we came to get the fineness value as 7%, which is higher than cement.

4.3 Testing of Quarry Dust

4.3.1 Specific Gravity Test

The specific gravity of quarry dust that we had got selected gives a value of 2.612. This value is slightly coincides with the specific gravity value of sand so that instead of sand we have used quarry dust.

4.3.2 Sieve analysis

The fineness value of quarry dust is calculated by sieve analysis process with the use of graph we have got the uniformity coefficient value as 2 and coefficient of curvature as 1.125

V. RESULT AND DISCUSSION

The results obtained are as discussed below

5.1 Compressive Strength Test

SET-1 Load Calculation's For SET 1 for 7day's

Sr.no	Weight of Cube grams	Load in KN	Area	Compressive Strength
1	2100	110	230x100mm^2	4.78N/mm^2

Load Calculation's For SET 1 for 14 day's

Sr.no	Weight of Cube grams	Load in KN	Area	Compressive Strength
1	2200	130	230x100mm^2	5.6N/mm^2

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SET-2 Load Calculation's For SET 2 for 7day's

Sr.no	Weight of Cube Grams	Load in KN	Area	Compressive Strength
1	1570	65	230x100mm^2	2.82N/mm^2

Load Calculation's For SET 2 for 14 day's

Sr.no	Weight of Cube Grams	Load in KN	Area	Compressive Strength
1	1678	70	230x100mm^2	3.04N/mm^2

SET-3 Load Calculation's For SET 3 for 7day's

Sr.no	Weight of Cube Grams	Load in KN	Area	Compressive Strength
1	1840	70	230x100mm^2	3.04N/mm^2

Load Calculation's For SET 3 for 14 day's

Sr.no	Weight of Cube Grams	Load in KN	Area	Compressive Strength
1	1978	75	230x100mm^2	3.26N/mm^2

SET- 4 Load Calculation's For SET 4 for 7day's

Sr.no	Weight of Cube Grams	Load in KN	Area	Compressive Strength
1	1670	50	230x100mm^2	2.17N/mm^2

Load Calculation's For SET 4 for 14 day's

Sr.no	Weight of Cube Grams	Load in KN	Area	Compressive Strength
1	1780	55	230x100mm^2	2.39N/mm^2

The Following Compressive Strength Test determine by as per IS-3495(part1)1992

5.2 Water Absorption Test

Dry weight of sludge brick = 2.5 Kg

Wet weight of sludge brick = 2.92 Kg

Water absorption = (2.92 – 2.5/ 2.5) X 100 = 16.8 %

VI. CONCLUSION

The conclusion is based on different sets of proportion, material and experimental sets are used and its tests is as follows, The water treatment plant sludge (WTP) is a best replacement in conventional brick or cement brick with sludge waste with high chrome content.

The researched brick type will be a big competitor to the cement brick and clay brick type in the market.

The maximum value of compressive strength was obtained in the 20% of sludge replacement in bricks.

Set 1 (60% quarry dust, 20% cement and 20% sludge) is best suitable for the structural applications.

Set 2 (60% quarry dust, 10% cement and 30% sludge) is best suitable for non-structural applications.

Set 3 (50% quarry dust, 20% cement and 30% sludge) it is also suitable for non-structural applications.

Set 4 (20% quarry dust, 30% cement and 50% sludge) will not be suitable for both structural and non-structural applications.

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4.1 Testing of Cement

4.1.1 Fineness Test:

The fineness of cement is calculated by passing the cement through 90mm sieve. The dry shrinkage cracks will easily form when the fineness increases.

Fineness of cement = (W1- W2) /W1 X 100

= 6 %

4.1.2 Standard Consistency Test

Co Standard consistency test is the amount of water required to prepare the plastic mix. It is used to find quantity of water for mixing with cement. The consistency of this cement is 33%

Sr. No.	% of water added to cement	Quantity of water added	Initial reading	Final reading
1	25	100	50	40
2	26	4	50	39
3	27	4	50	38

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4	28	4	50	36
5	29	4	50	29
6	30	4	50	20
7	31	4	50	16
8	32	4	50	10
9	33	4	50	6

4.1.3 The Setting Time Test

- Initial setting test
- Final setting test

4.1.4 Specific Gravity Test

With the apparatus density bottle and weighing balance, the following procedure was carried out to determine the specific gravity of cement. 100gm of cement is weighted 990 ml of kerosene is filled in a specific gravity bottle. sample of cement is placed on specific gravity bottle, till the level of kerosene reaches 100ml mark the quality of cement placed in the bottle is calculated. Then the specific gravity of cement is found out by using he following reaction specific gravity of cement =weight of cement (volume of 10ml by weight of kerosene of equal volume of cement.

Specific gravity of cement = 3.19

4.2 Testing of Sludge

4.2.1 Specific Gravity Test

The same procedure was followed to determine the specific gravity of sludge. Instead of cement we used sludge in that procedure.

Specific gravity of sludge = W5X (W3- W1)/ (W5+W3- W4) X (W2- W1)

by calculating using the formula we got specific gravity of sludge as 2.90

4.2.2 Fineness Test

The fineness test was done for the dry sample of sludge. 100gms of sludge was sieved horizontally for about 10 to 15 min and then residues in each sieve was weighed. From that we came to get the fineness value as 7%, which is higher than cement.

4.3 Testing of Quarry Dust

4.3.1 Specific Gravity Test

The specific gravity of quarry dust that we had got selected gives a value of 2.612. This value is slightly coincides with the specific gravity value of sand so that instead of sand we have used quarry dust.

4.3.2 Sieve analysis

The fineness value of quarry dust is calculated by sieve analysis process with the use of graph we have got the uniformity coefficient value as 2 and coefficient of curvature as 1.125

V. RESULT AND DISCUSSION

The results obtained are as discussed below

5.1 Compressive Strength Test

SET-1 Load Calculation's For SET 1 for 7day's

Sr.no	Weight of Cube grams	Load in KN	Area	Compressive Strength
1	2100	110	230x100mm^2	4.78N/mm^2

Load Calculation's For SET 1 for 14 day's

Sr.no	Weight of Cube grams	Load in KN	Area	Compressive Strength
1	2200	130	230x100mm^2	5.6N/mm^2

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SET-2 Load Calculation's For SET 2 for 7day's

Sr.no	Weight of Cube Grams	Load in KN	Area	Compressive Strength
1	1570	65	230x100mm^2	2.82N/mm^2

Load Calculation's For SET 2 for 14 day's

Sr.no	Weight of Cube Grams	Load in KN	Area	Compressive Strength
1	1678	70	230x100mm^2	3.04N/mm^2

SET-3 Load Calculation's For SET 3 for 7day's

Sr.no	Weight of Cube Grams	Load in KN	Area	Compressive Strength
1	1840	70	230x100mm^2	3.04N/mm^2

Load Calculation's For SET 3 for 14 day's

Sr.no	Weight of Cube Grams	Load in KN	Area	Compressive Strength
1	1978	75	230x100mm^2	3.26N/mm^2

SET- 4 Load Calculation's For SET 4 for 7day's

Sr.no	Weight of Cube Grams	Load in KN	Area	Compressive Strength
1	1670	50	230x100mm^2	2.17N/mm^2

Load Calculation's For SET 4 for 14 day's

Sr.no	Weight of Cube Grams	Load in KN	Area	Compressive Strength
1	1780	55	230x100mm^2	2.39N/mm^2

The Following Compressive Strength Test determine by as per IS-3495(part1)1992

5.2 Water Absorption Test

Dry weight of sludge brick = 2.5 Kg

Wet weight of sludge brick = 2.92 Kg

Water absorption = (2.92 – 2.5/ 2.5) X 100 = 16.8 %

VI. CONCLUSION

The conclusion is based on different sets of proportion, material and experimental sets are used and its tests is as follows, The water treatment plant sludge (WTP) is a best replacement in conventional brick or cement brick with sludge waste with high chrome content.

The researched brick type will be a big competitor to the cement brick and clay brick type in the market.

The maximum value of compressive strength was obtained in the 20% of sludge replacement in bricks.

Set 1 (60% quarry dust, 20% cement and 20% sludge) is best suitable for the structural applications.

Set 2 (60% quarry dust, 10% cement and 30% sludge) is best suitable for non-structural applications.

Set 3 (50% quarry dust, 20% cement and 30% sludge) it is also suitable for non-structural applications.

Set 4 (20% quarry dust, 30% cement and 50% sludge) will not be suitable for both structural and non-structural applications.

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Analysis of G+4 building structure for Seismic Retrofitting using Cross Bracing

Mr. Ajay P. Shinde[1], Mr. Shubham S. Khomane[2], Mr. Shubham M. Khade[3],Mr. Aniket A. Shelar[4], Mr. Heramb S. Chavan[5], Mr. Shubham H. Pisal[6], Mr. A. S. Shah[7]

[12345]Students, Department of Civil Engineering, YSPM's Yashoda Technical Campus, Satara
[6]Assistant Professor, Department of Civil Engineering, YSPM's Yashoda Technical Campus, Satara

Abstract
As the earthquakes are an inconsistent phenomenon they may or mostly may not occur in entire lifespan of building, Designing abuilding structure to sustain during an earthquake makes it very uneconomical, Hence a Structural model is going to be used for comparison in between building structure models with seismic retrofitting techniques such as Steel Bracings. In this paper a G+4 building structural model is analysed in zone III and zone IV by using Arduino Earthquake Detector Alarm with Seismic Graph using Accelerometer. Various characters like consistency, lateral displacement and storey drift will be studied. The main aim of this paper is to compare the differences in Structural model without Steel Bracing and Structural model with Steel Bracings with help of different levels applied to the model with increase in force applied from zone III to zone IV.
Keywords: *Structural Model, Arduino Earthquake Detector Alarm, Accelerometer, Seismic Graph.*

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I. INTRODUCTION

In the past thirty years, moderate to severe earthquakes occurs around the world every year. Such events lead to damage to the concrete structures as well as failures. Thus, the aim is to Focus on a few specific procedures which may improve the practice for the evaluation of seismic vulnerability of existing reinforced concrete buildings of more importance and for their seismic retrofitting by means of various innovative techniques such as base isolation and mass reduction. So Seismic Retrofitting is a collection of mitigation technique for Earthquake engineering. It is of utmost importance for historic monuments, areas prone to severe earthquakes and tall or expensive structures. The existing building stock poses a much more serious and complex seismic safety problem when compared to safe earthquake design of new construction. The vast majority of structures located in seismic areas exhibit deficiencies in their resistance to earthquake loads due to a number of reasons, highlighted below.

Older construction, designed according to earlier codes, may not comply with current seismic regulations since focus used to be primarily on warranting sufficient capacity for gravity loads alone. Moreover, the past thirty years have witnessed such a significant increase of knowledge in the field of earthquake engineering that even relatively modem structures may no longer meet the prerequisites of constantly-developing regulations. As a result, several shortcomings can be found in existing buildings such as irregular structural configuration, inappropriate member detailing for ductility and insufficient lateral stiffness, amongst others.All the above considered, it seems clear that repair and strengthening of both old structures designed according to outdated codes and new but defective earthquake-resistant construction, is urgently needed. This requirement also arises where existing structures must comply with more recent code stipulations, or when these structures are to be reassessed for higher loads.

1.1 Seismology

Seismology is the scientific study of earthquakes and the propagation of elastic waves through the Earth or through other planet-like bodies. The field also includes studies of earthquake environmental effects such as tsunamis as well as diverse seismic sources such as volcanic, tectonic, glacial, fluvial, oceanic, atmospheric, and artificial processes such as explosions. A related field that uses geology to infer information regarding past earthquakes is paleo-seismology. A recording of Earth motion as a function of time is called a seismogram. A seismologist is a scientist who does research in seismology.

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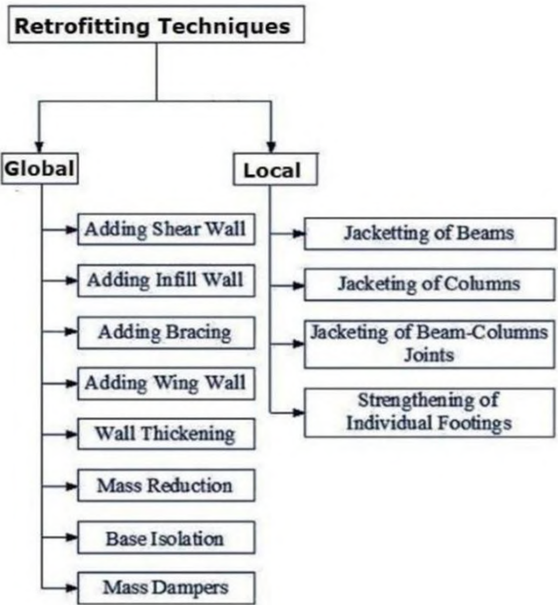
National Centre for Seismology (NCS) is the nodal agency of the Government of India for monitoring earthquake activity in the country. NCS maintains the National Seismological Network of more than 150 stations each having state of art equipment and spreading all across the country NCS monitors earthquake activity all across the country through its 24x7 around-the-clock monitoring centre. NCS also monitors earthquake swarm and aftershock by deploying a temporary observatory close to the affected region

1.2 Classification



Above mentioned are various types of Seismic Retrofitting Techniques used both locally and on Global level. Seismic Retrofitting Techniques are required for concrete constructions which are vulnerable to damage and failures by seismic forces. In the past thirty years, moderate to severe earthquakes occurs around the world every year.Thus the aim is to Focus on a few specific procedures which may improve the practice for the evaluation of seismic vulnerability of existing reinforced concrete buildings of more importance and for their seismic retrofitting by means of various innovative techniques such as base isolation and mass reduction. It is of utmost importance for historic monuments, areas prone to severe earthquakes and tall or expensive structures. In this project we will be dealing with the most commonly used Seismic Retrofitting technique known as Cross Bracing.

1.3 Cross Bracing

Bracing is a very effective global upgrading strategy to enhance the global stiffness and strength of steel and composite frames (Fig 02). It can increase the energy absorption of structures and/or decrease the demand imposed by earthquake loads. Structures with augmented energy dissipation may safely resist forces and deformations caused by strong ground motions. Generally, global modifications to the structural system are conceived such that the design demands, often denoted by target displacement, on the existing structural and non-structural components, are less than their capacities (Fig 02). Lower demands may reduce the risk of brittle failures in the structure and/or avoid the interruption of its functionality. The attainment of global structural ductility is achieved within the design capacity by forcing inelasticity to occur within dissipative zones and ensuring that all other members and connections behave linearly.

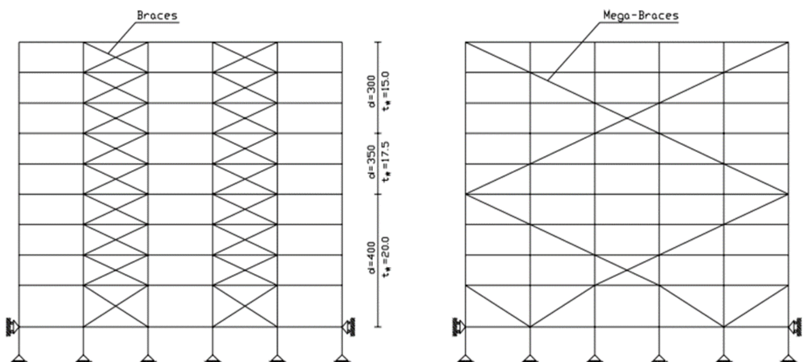


Figure 1:Layout of braced frames: concentrically- (left) and mega-braced (right) frames.

There are different types of Cross Bracing Techniques which are as follows:
1) Concentric based frames (CBFs)
2) Eccentric based frames (EBFs)
3) The novel knee-base frames (KBFs)

Concentric based frames are further classified in following types:
(i) V – Type Cross Bracing
(ii) X – Type Cross Bracing
(iii) K – Type Cross Bracing
(iv) Opposite V – Type Bracing
(v) Diagonal Bracing
(vi) 2 Storey X – Bracing.

In this project we will be dealing with V – Type and X – Type Cross Bracing.

1.4 Seismic Zones
Different Seismic Zones in India
(i) Seismic Zone II: Zone II is classified as the low-damage risk zone.
(ii) Seismic Zone III: Seismic Zone 3/III is classified as the moderate-damage risk zone.
(iii) Seismic Zone IV: Zone IV is considered the high-damage risk zone.
(iv) Seismic Zone V: Zone V has the highest risk of damaging earthquakes.

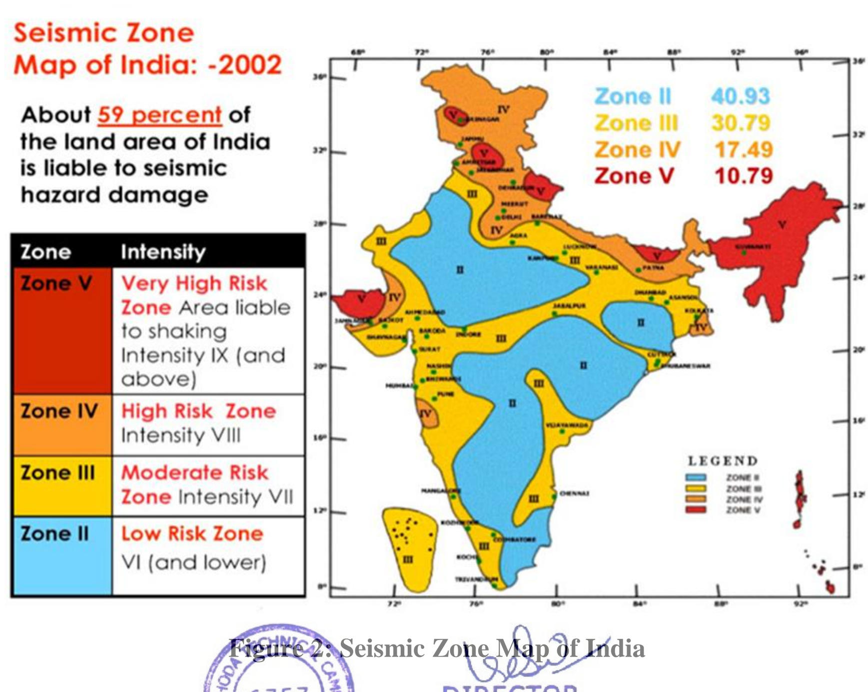


Figure 2: Seismic Zone Map of India

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According to the seismic zoning map of the country, India is divided into four seismic zones. Also known as earthquake zones, these seismic zones are formed on the basis of scientific inputs related to the following:

(i) The Seismicity or the Frequency of Earthquakes in a Region
(ii) Earthquakes That Have Hit the Country in the Past The four zones of earthquake in India, as discussed below:
(iii) Seismic Zone II: Zone II is classified as the low-damage risk zone. This is the least seismically active zone, meaning the areas that fall under these zones in India have a low chance of having an earthquake. Zone II covers earthquake-prone areas, which are 41% of India. Here, the Indian Standard (IS) Code allots a zone factor of 0.10.
(iv) Seismic Zone III: Seismic Zone 3/III is classified as the moderate-damage risk zone. Here, the IS Code allots 0.16 to this zone. Zone III, or moderate earthquake zone, covers 30% of India.
(v) Seismic Zone IV: Zone IV is considered the high-damage risk zone. The IS Code allots 0.24 to this zone. Moreover, 18% of the total area of the country belongs to Zone IV.
(vi) Seismic Zone V: Zone V has the highest risk of damaging earthquakes. The IS Code has assigned a factor of 0.36 for this very high-risk damage zone. Around 11% of India falls under Zone V.
Note: There are no cities in India which fall under Seismic Zone I

The above-mentioned list of earthquake zones in India gives a comprehensive knowledge of the different zones and total areas they cover. Let us now take a look at the top 10 cities prone to an earthquake.

Table 1: Magnitudes & Intensity of Earthquake Globally

Magnitude	Description	Intensity (Mercalli)	Average Frequency of Occurrence Globally
1.0 – 1.9	Micro	I	Several Million Per year
2.0 – 2.9	Minor	I to II	Over one million per year
3.0 – 3.9		III to IV	Over 1,00,000 per year
4.0 – 4.9	Light	IV to VI	10,000 to 15,000 per year
5.0 – 5.9	Moderate	VI to VII	1,000 to 1,500 per year
6.0 – 6.9	Strong	VIII to X	100 to 150 per year
7.0 – 7.9	Major	X or greater	10 to 20 per year
8.0 – 8.9	Great	X or greater	One per year
9.0 & greater		X or greater	One per 10 to 50 years

1.5 Satara District Seismic Zones

(i) As Satara District comes under earthquake prone areas it mainly gets divided into two different zones.
(ii) It gets divided into Zone III and Zone IV respectively.
(iii) As Zone III is also called as Very strong intensity zone its intensity on MMI scale is around “VII”.
(iv) As Zone IV is called as Severe Intensity zone its intensity on the MMI scale is around “VIII”.
(v) Hence, as for Satara District region the typical MMI (Modified Mercalli Intensity) is around VII & VIII.
(vi) Which then comes under the Magnitude of Earthquake as in 6.0 to 7.0.

Table 2: Magnitude vs. MMI Scale

Magnitude	Typical MMI
1.0 – 2.9	I
3.0 – 3.9	II – III
4.0 – 4.9	IV – V
5.0 – 5.9	VI – VII
6.0 – 6.9	VII – IX
7.0 & higher	VIII or higher

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III. OBJECTIVES

2.1 To Develop G+4 Building structure model with Cross Bracing.
2.2 To apply different levels of magnitudes to all models.
2.3 To compare between Building Structure models of both buildings with Cross Bracing and without Cross Bracing.
2.4 To Obtain result and conclusion based on testing, that demonstrates the validity of the designed technique.

IV. METHODOLOGY

As the earthquakes are an inconsistent phenomenon they may or mostly may not occur in entire lifespan of building, Designing a building structure to sustain during an earthquake makes it very uneconomical, Hence a Structural model is going to be used for comparison in between building structure models with seismic retrofitting techniques such as Steel Bracings. In this paper a G+4 building structural model is analysed in zone III and zone IV by using Arduino Earthquake Detector Alarm with Seismic Graph using Accelerometer. Various characters like consistency, lateral displacement and storey drift will be studied. The main aim of this paper is to compare the differences in Structural model without Steel Bracing and Structural model with Steel Bracings with help of different levels applied to the model with increase in force applied from zone III to zone IV.

Bracing play important role in keeping structure stable. Earthquake produces inertial forces in structure. These inertial forces act in the form of base shear on structure. Base shear is distributed to different floor along the height of the building. This force produces later displaces in structure. For high rise building, lateral displacements are common due towing loading. But if the earthquake is of high intensity, it can be disastrous. Bracings play important role in distributing this force in columns and beams. In this project we have analysed unbraced structure with structures having different bracings.

X-bracing system has shown good results when it comes to reducing lateral displacements. Base shear values are same in both directions. Since number of bracings along X-directions were more, bracings shown good performance in lateral displacements along X-axes. Diagonal bracing shows overall good performance considering maximum bending moment. V-bracing has shown good performance considering Maximum support Reactions. Weight of the structure remains almost same. Not more than 2 percent change in weights of structure. Since base shear is dependent on weight, base shear also remain similar.

V. TESTING AND CONVERGENCE

1. As for Amplitudes regarding in the application in Project Model.
2. There will be levels used for change in amplitude of earthquake.
3. i. 1 Hz to 20 Hz in 30 seconds amplitude 0.50 as for the Shake in Platform Level 1, we will be using Total Shake.
   ii. Level 2 - 0.75 amplitude change in every 30 seconds from 20 Hz to 40 Hz.
   iii. Level 3 – 1.00 amplitude change in every 30 sec.
4. We will also be adding pulse motions for sudden jerks in the structural model.
5. Then we will be increasing the amplitudes on the shake table till the building with no bracing collapse.
6. After the collapse of the building with no bracing, we will be taking results from the other two building structures.
7. We will take different results for both building structure models with X-Type and V-Type bracing to check its durability with increase in the amplitudes simultaneously.
8. As the project model is based on the Satara district seismic zone, the earthquake zones will be Zone III and Zone IV.
9. Hence, we will need to take results up to the magnitude of amplitude level 7.5 to 8.0 as per the severe intensity zone in the MMI scale.

VI. CONCLUSION

Lateral forces are distributed to beams and columns by bracings. In this project a comparative analysis of unbraced structure with structures having different bracings. With parameters such as Bending Moments, Lateral displacements, support reactions. X-bracing system has shown good results when it comes to reducing lateral displacements.

On the basis of the present study, following conclusions are made:

- As per displacement criteria, bracings are good to reduce the displacement and in case of X and V-bracing, the displacement is higher than without bracing because of irregularity in shape of the structure.
- The reactions and weight of the structure are more in different types of bracing structures when compared to un braced structure with same configuration of the structure.
- It is also seen that as there are different bracing systems employed the displacement and storey drifts, may increase or decrease for the braced buildings with the same configurations.

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- The braced buildings of the storey drift either increases or decreases, as compared to un braced building with the same configuration for the different bracing system.

VII. FUTURE SCOPE

This project primarily focused on concentric bracings. There are so many different types of concentric bracings. In this project only four of them are utilized. There are various types of eccentric bracings too. Eccentric bracings can useful when lateral loads are of know directions. In future works this analysis can be utilized as a source of data for further analysis. There could be multiple arrangements. Here we have only focused on only one type of arrangements. This work can be further carried out with different arrangements. Bracing types can be compared by using many more parameter

This project can also be tested for dynamic loading, wind loads. Work is done on static coefficient method. It can be redone using Response spectra method, Time history analysis. This is a symmetrical structure. Further projects can be done on irregular structures. Irregularity can induce unexpected forces in structure.

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Comparative Study of Behavior of Framed Structure Under Seismic Zone III & IV Using STAAD Pro

Mr. Girish S. Gaikwad[1], Mr. Sarang P. Patankar[2], Mr. Arjun M. Shinde[3], Mr. Rohan N. Saste[4], Mr. Siddhant A. Nikam[5], Mr. A. N. Shaikh[6]
[12345]Students, Department of Civil Engineering, YSPM's Yashoda Technical Campus, Satara
[6]Assistant Professor, Department of Civil Engineering, YSPM's Yashoda Technical Campus, Satara

Abstract
Designing a structure to sustain during an earthquake makes it very uneconomical, as the earthquakes may or mostly may not occur in entire lifespan of building since it is inconsistent phenomena. In this paper a G+4 RCC building is designed in zone III and zone IV by using STAAD Pro software. Various characters like lateral displacement and storey drift will be studied. The main aim of this paper is to think on variations in RCC members, most extreme shear power, greatest redirection all these factors shows increase from zone III to zone IV.
Keywords: *Seismic zones, STAAD PRO, Lateral Displacement, .*

Date of Submission: 05-06-2022 Date of acceptance: 20-06-2022

I. INTRODUCTION

Designing structure with the help of STAAD Pro V8i which is referenced to IS 1893(PART 1): 2002 "Criteria for Earthquake Resistant Design of Structure" ensures that building has minimum strength to withstand minor earthquake occurring frequently and resisting moderate earthquakes without significant structural damage. This document is presented to improve the productivity of sustained earthquake mitigation strategies and the capacity to secure structures, frameworks, to Investigate a multiplex RCC operating for open shaking strength to think about the effects of different seismic zones, Knowing the relationship between different procedures for seismic inspection and their seismic response, gain useful learning in basic inspection, seismic assessment, drafting and identification of auxiliary parts using earthquake resistant design norms. We are also configuring the G+4 custom build, it means that if the zone changes from zone III to zone IV, the structure planned by us at that point will be fixed. Also, by calculating this we will perceive the amount spent putting together such a structure.

Seismic tremor shaking is irregular and varies with time. Be that as it may, most plan codes speak of inertia forces caused by jolting as the net effect of arbitrary jolts, such as static parallel power proportional to the structure. This strength is called the seismic design base shear VB and remains the base quantity associated with the strength-based earthquake resistant structure of structures. This strength is based on the seismic hazard in the area of the structure spoken by the seismic zone factor z. The codes reflect this by presenting a flexibility factor sa/g. This way of thinking is presented with the help of the response reduction factor r, which is larger for flexible structures and smaller for weak structures. Therefore, the seismic shake claim plan is evaluated solely on the basis of probabilistic ideas and the earthquake effects plan is called a seismic shake safe structure against reasonable estimate of interest. The design base shear VB was taken according to the Indian seismic code is 1893(part 1)-2007.

1.1 Basic Design Codes

Design should be carried so as to confirm to to the following:
1. IS 456: 2000- Plain and reinforced concrete- code of practice (fourth revision)
2. National Building Code 2005
3. Loading Standards IS 875 (Part 1-5): 1987- code of practice for design loads (other than earthquake) for buildings and structures (second revision)
Part 1: Dead Loads
Part 2: Live Loads
Part 3: Wind Loads
Part 4: Snow Loads

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Part 5: Special Loads and load combinations

1.2 Design Handbooks

SP 16: 1980- Design Aids (For RCC) to IS 456: 1978
SP 24: 1983- Explanatory handbook on IS 456:1978
SP 34: 1987- Handbooks on concrete Reinforced and Detailing.

1.3 STAAD Pro. V8i

Structural Analysis & Design is used to create the model which would then be able to investigated, analysed & designed. After examination and configuration is finished, the GUI can likewise be utilised to see outcomes graphically. It is a general useful census for auxiliary inspection and combines of Steel, concrete, Timber and aluminum construction. Its adaptability for different codes of design makes it versatile.

II. OBJECTIVES

2.1 To design G+ 4 structure for zone III & IV on STAAD Pro.
2.2 To compare the behavior of framed structure in seismic zone III & IV.
2.3 To make a total plan of the main auxiliary components of a specific structure & find out steel increment.

III. METHODOLOGY

3.1 Creation of node foci: Considering the centreline layout of the plan, we entered the hub documents into the STAAD document.
3.2 Representation of bars and segments: Using the inclusion bar layout, we plotted between beams & columns.
3.3 3D perspective on the building: Here we used the transition repetitive pattern in the Y header to get a 3D perspective on the structure.
3.4 Supports and property: After the formation of the structure, the supports at the base of the structure are specified as fixed. Likewise, the Materials were determined and the cross segments were distributed to the individuals.
4 **3D render view:** After feature clustering, a 3D rendering perspective can be viewed on the structure.
5 **Assignment of seismic loads:** We have defined the seismic loads specified in the IS1893:2002 code with appropriate ground loads in order to disable seismic loads instantly. Loads are included load case subtleties in +X, -X, +Z, -Z headings with determined seismic factor.
6 **Assignment of wind loads:** Wind loads are characterized according to IS 875 Part 3, depending on the determined power and input factor.
7 **Assignment of dead loads:** For external dividers, internal dividers, parapet dividers, constant loads including the self-weight of the structure are determined in accordance with IS 875 part 1.
8 **Assignment of live loads:** Live loads are relegated for each floor as 3 KN/M^2 dependent on IS 875 PART 2.
9 **Adding of load combination:** After all batches have been dropped, batch mixes are given with the appropriate factor of safety in accordance with IS 875 Part 5.
10 **Analysis:** After all the above progress paid off, we played out examination and checked for errors.
11 **Design:** Finally, the solid plan proceeds according to IS 456:2000, characterizing the appropriate plan orders for the various key segments. After the allocation of orders, we investigated whether there were errors again concrete design.
12 **Report:** After no error found the reports are downloaded and same procedure is repeated but this time with different Seismic Zone.
After following the above specifications the structure is designed for the Seismic zone III. Since, the same structure can be designed for Zone IV only with minor alterations in the Seismic Load case and reports can be compared.

IV. SIMULATION

The input data is as follow,
1.START CONCRETE DESIGN
2.CODE INDIAN
3.CLEAR 0.025 MEMB 124 125 127 TO 172 174 TO 185 189 191 195 197 TO 263 280 - 228. 281 TO 344 360 TO 424 440 TO 504 520 TO 584 229.
3.CLEAR 0.04 MEMB 81 84 88 92 93 96 97 102 112 116 TO 118 186 190 192 196 264 - 230. 265 TO 276 278 279 345 TO 359 425 TO 439 505 TO 519 585 TO 604 231.

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4.FYMAIN 415000 ALL
5.FYSEC 415000 ALL
6.MAXMAIN 32 ALL
7.MAXSEC 16 ALL
8.MINMAIN 8 ALL
9.MINSEC 8 ALL
10.RATIO 4 MEMB 81 84 88 92 93 96 97 102 112 116 TO 118 186 190 192 196 - 238. 264 TO 276 278 279 345 TO 359 425 TO 439 505 TO 519 585 TO 604 239.
11.DESIGN BEAM 124 125 127 TO 172 174 TO 185 189 191 195 197 TO 263 280 TO 344 - 240. 360 TO 424 440 TO 504 520 TO 584

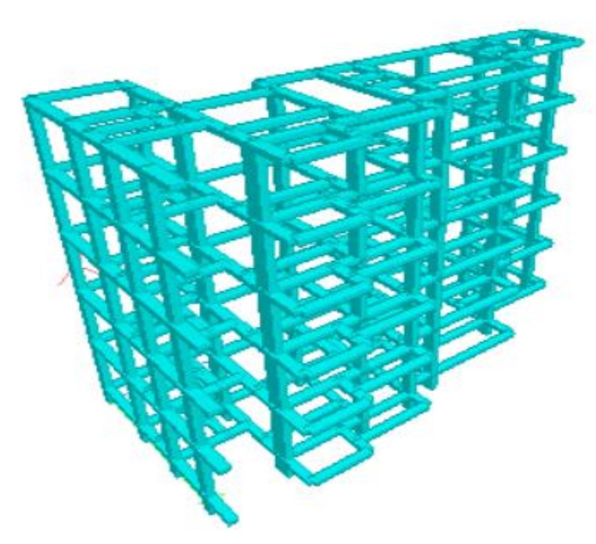

Figure1: 3-D Rendered View

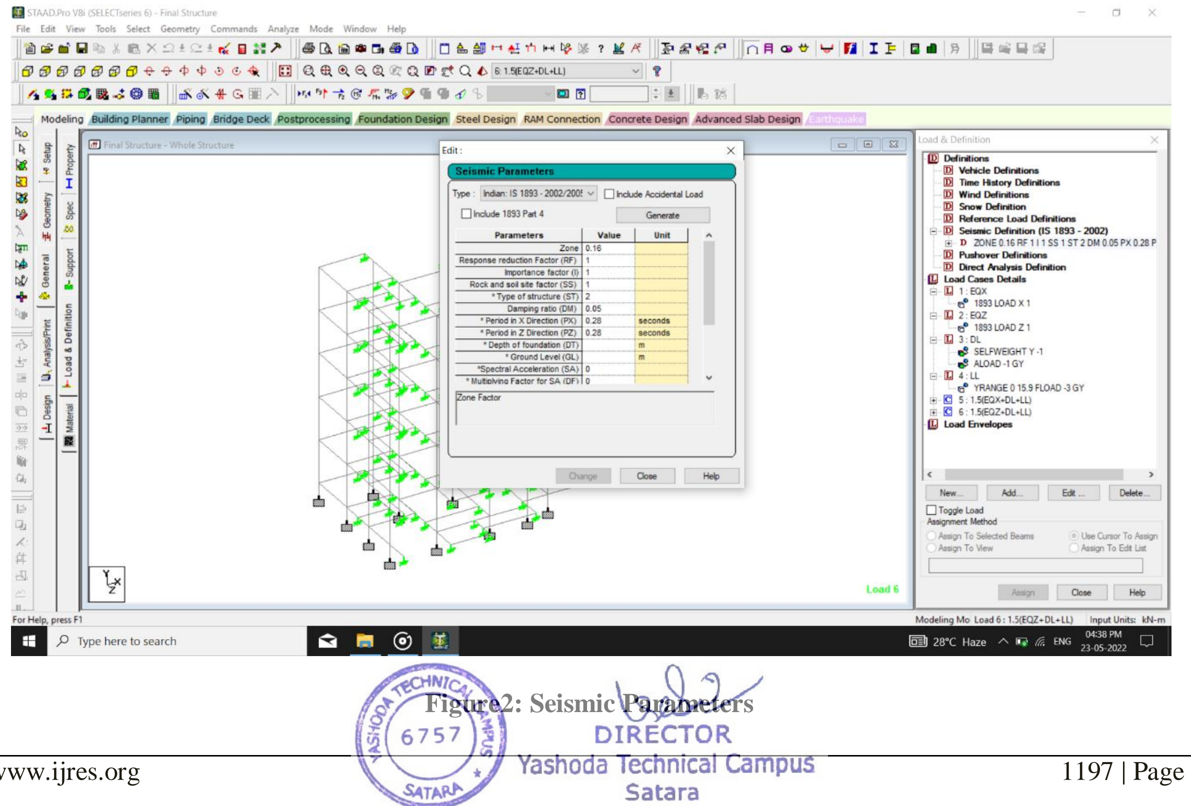

Figure2: Seismic Parameters

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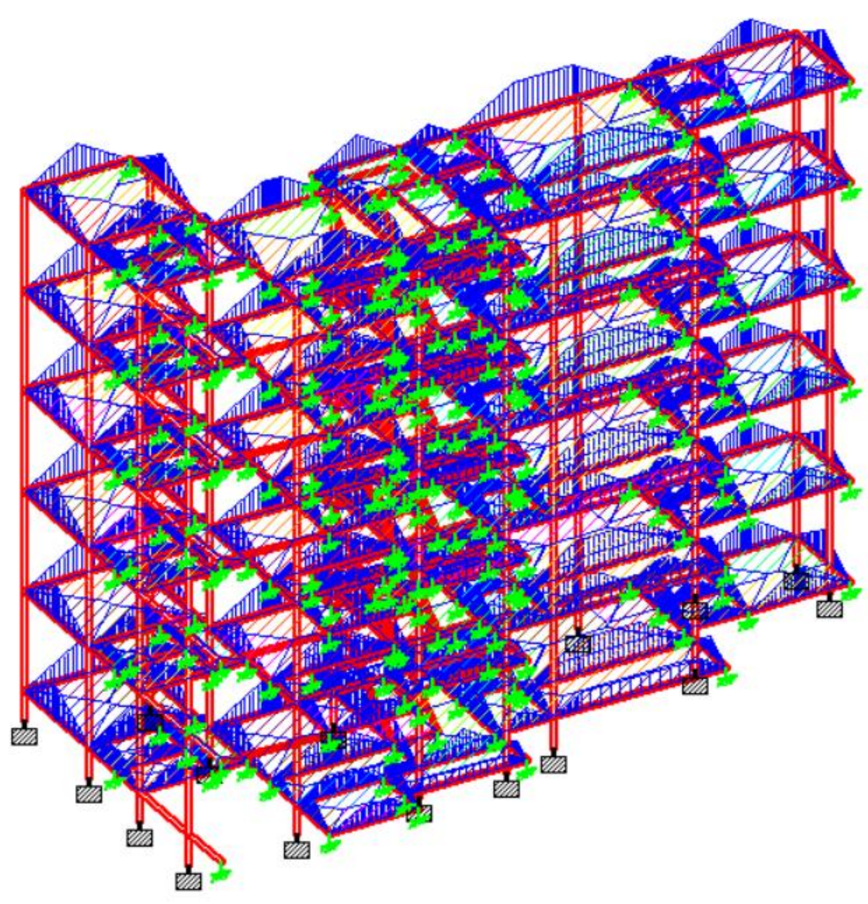

Figure3: Dead Load & Live Loads

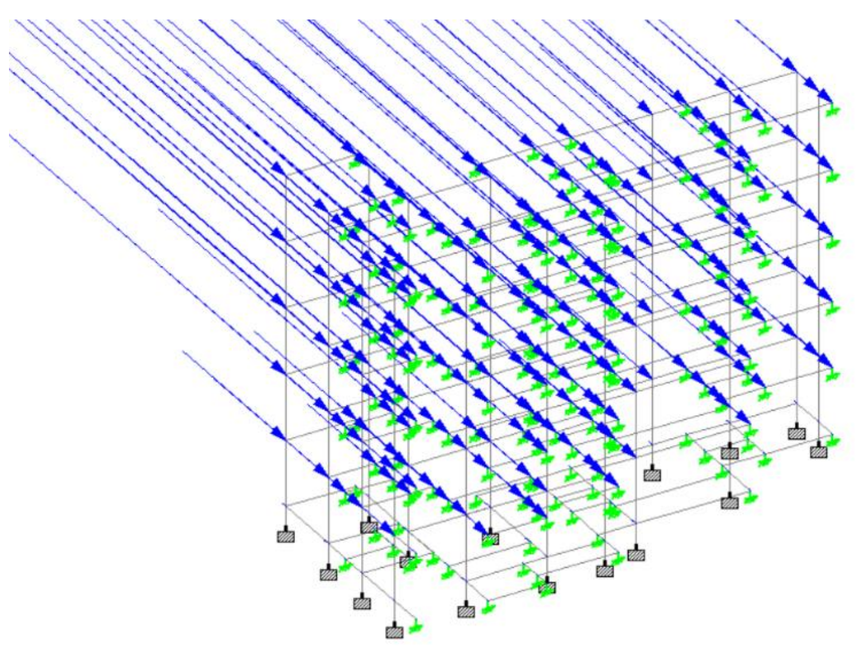

Figure4: Seismic Forces in X- Direction (maximum)

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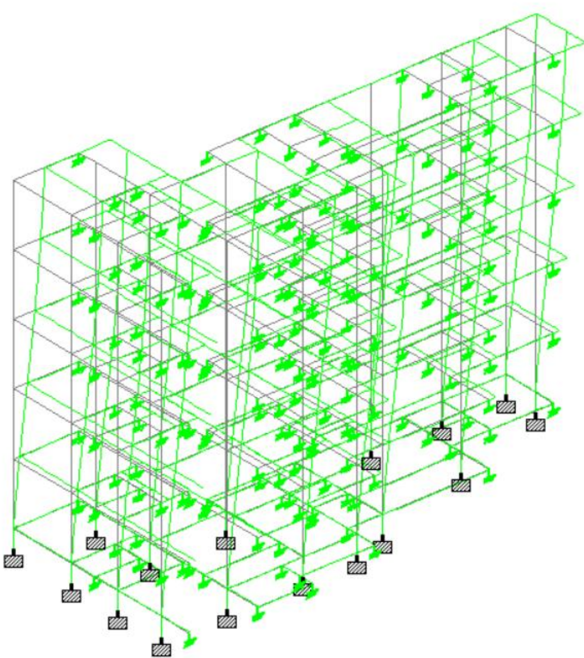

Figure5: Deflection Of Members

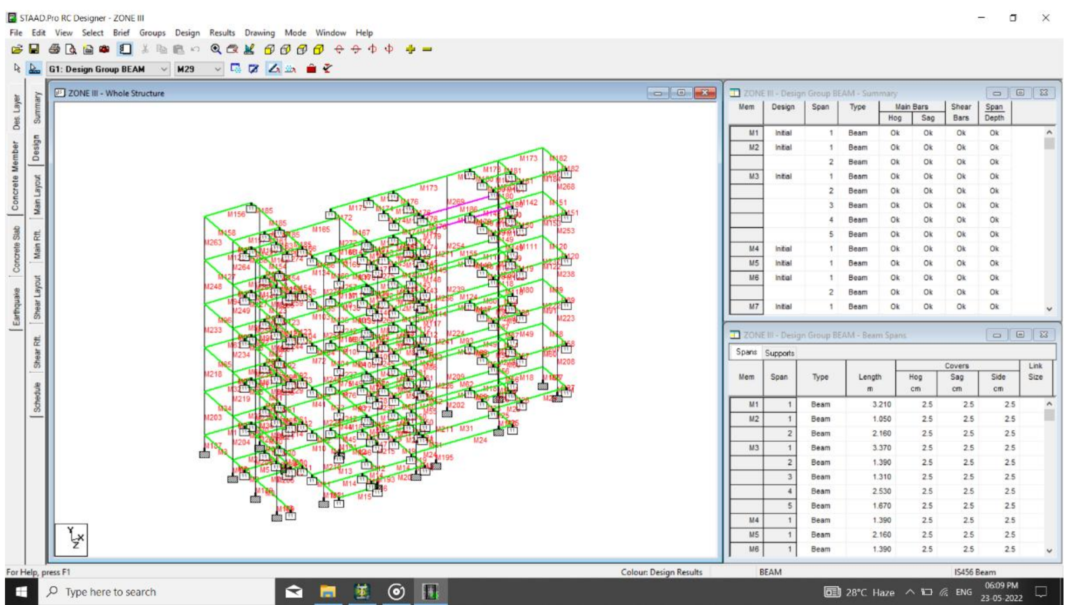

Figure6: Beam Check

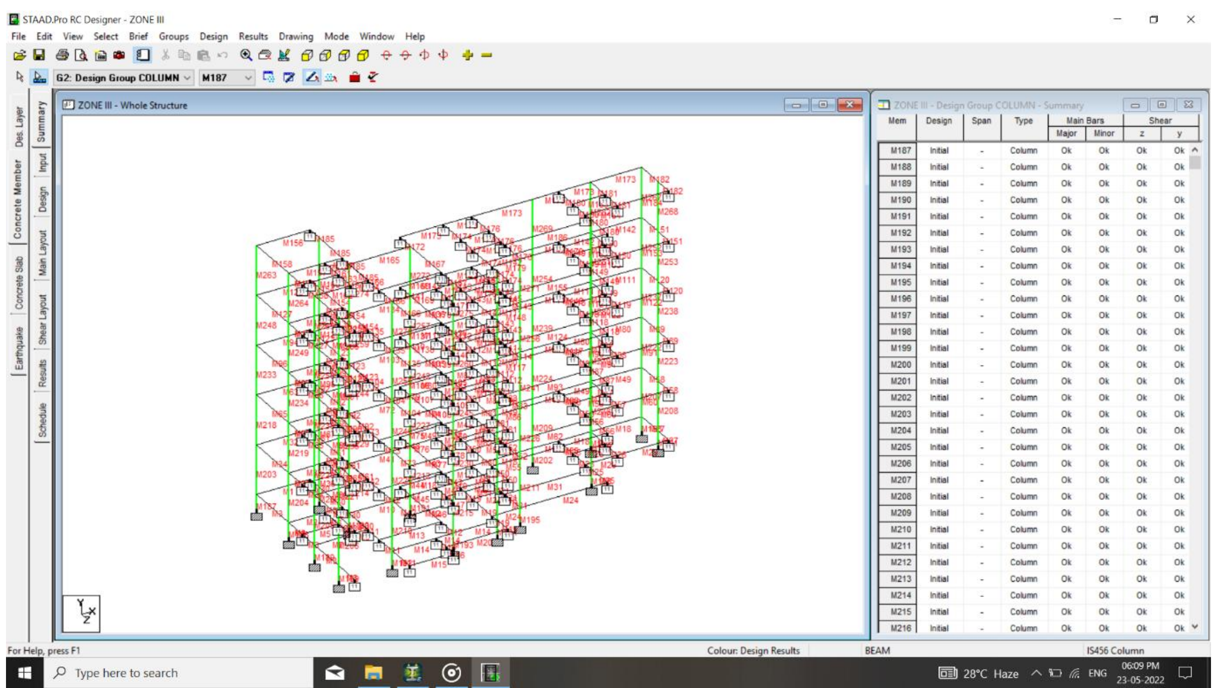

Figure7: Column Check

V. CONCLUSION

5.1 Total 2.47% more steel is require to design the structure from Zone III to Zone IV.
5.2 Maximum nodal displacement is increased by 8.33mm showing more horizontal forces in higher zone.
5.3 Maximum bending moment is increased by 30.84 kNm results in more steel in beam section.
5.4 Maximum shear forces increased by 15.32 kN resulting in additional 1.3% shear reinforcement in zone IV.
5.5 After analyzing the G+4 storey building structure, it was concluded that the building is safe under dead load, wind load and seismic loads in both zones if additional 2.5% reinforcement is provided.
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ABSTRACT

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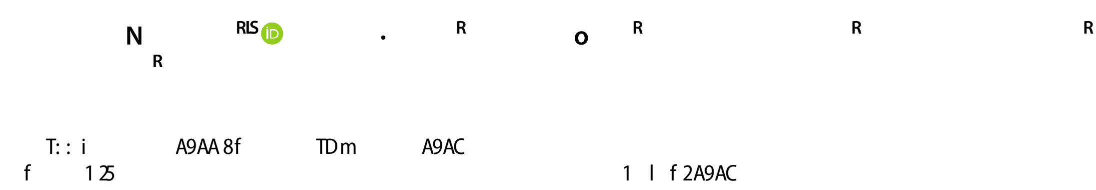

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Yashoda Technical Campus
Satara

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Fake news detection in social media based on sentiment analysis using classifier techniques

Sarita V Balshetwar[1,2] **· Abilash RS**[3] **· Dani Jermisha R**[4]

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Abstract

Fake news on social media, has spread for personal or societal gain. Detecting fake news is a multi-step procedure that entails analysing the content of the news to assess its trustworthiness. The article has proposed a new solution for fake news detection which incorporates sentiment as an important feature to improve the accuracy with two different data sets of ISOT and LIAR. The key feature words with content's propensity scores of the opinions are developed based on sentiment analysis using a lexicon-based scoring algorithm. Further, the study proposed a multiple imputation strategy which integrated Multiple Imputation Chain Equation (MICE) to handle multivariate missing variables in social media or news data from the collected dataset. Consequently, to extract the effective features from the text, Term Frequency and Inverse Document Frequency (TF-IDF) are introduced to determine the long-term features with the weighted matrix. The correlation of missing data variables and useful data features are classified based on Naïve Bayes, passive-aggressive and Deep Neural Network (DNN) classifiers. The findings of this research described that the overall calculation of the proposed method was obtained with an accuracy of 99.8% for the detection of fake news with the evaluation of various statements such as barely true, half true, true, mostly true and false from the dataset. Finally, the performance of the proposed method is compared with the existing methods in which the proposed method results in better efficiency.

✉ Sarita V Balshetwar
balshetwar.satara@gmail.com

Abilash RS
abilash.res2k21@yahoo.com

Dani Jermisha R
danijermisha01@yahoo.com

[1] YSPM'S, YTC, Faculty of Engineering, Satara, Maharashtra, India

[2] DBATU, lonere, Raigad, Maharashtra, India

[3] Bethlahem Institute of Engineering, Ulaganvillai, Tamil Nadu, India

[4] Arunachala College of Engineering For Women, Manavilai, Tamil Nadu, India

Keywords Sentiment analysis (NLP) · Fake news · Social media · Missing data · Multiple imputation · Naïve Bayes classifier · Deep neural network (DNN)

1 Introduction

People are spending more and more time interacting on social media, as the wide adoption of smartphones makes their access available almost anytime and anywhere, which is not the case with traditional media. In addition, they facilitate interaction with friends, families, and even strangers through the comment chains, be it through comments, discussions, or simply like and dislike buttons. This has made social media a main channel for the dissemination of news. However, new technologies and features can be used through social media platforms to spread fake news on a large scale. Such inaccurate information might result either from a deliberate attempt to deceive or mislead (disinformation) or from an honest mistake (misinformation). Rumours can fall into either of these two categories, depending on the intent of the source, given that rumours are not necessarily false but may turn out to be true. Unlike rumours, fake news is, by definition, always false and, thus, can be seen as a type of disinformation. Therefore, credible and reliable sources of information are needed so that the public does not fall prey to the intentions of those interested in manipulating reality.

Fake news can function as propaganda or misinformation, but it always appeals to the emotions of the public and the intent to cover rational responses, analysis, and comparison of information from several sources, encouraging inflammation and outrage and can easily lead to conspiracy theories and partisan biased content that negatively affects. The major source of news and data for the public is served by social media and online news articles because it is easily accessible, subsidized and readily available with one click. However, simultaneously, it also helps to spread false news that has significant negative effects on society, that is, messages that are deliberately misinformed. It has many similarities with spam messages since they share common features such as grammatical mistakes, false information, using a similarly limited set of words, and they contain emotionally coloured information that affects the reader's opinion [1]. To alleviate this problem, research on the identification of false news has gained more consideration recently. Despite the many computational solutions currently available to detect fake news, the lack of a comprehensive and community-based false news database has become one of the significant obstacles. Large-scale news passes over social media makes manual verification impossible, which promotes the design and implementation of automated systems for detecting fake news [10].

Detecting fake news is a layered process that involves analysis of the news contents to determine the truthfulness of the news. The news could contain information in various formats such as text, video, image, etc. Combinations of different types of data make the detection process difficult. In addition, raw data collected is always expected to be unstructured and contain missing values in the data. As fake news produces big, incomplete, unstructured, and noisy data [2], raw data pre-processing is extremely important to clean and structure the data before feeding it into detection models. Thereby, fake news creators use many new ideas to make their false creations successful, one of which is to stimulate the emotions of the beneficiaries. This leads to sentiment analysis, the portion of the analysis of the text is responsible for establishing the polarization and the emotional strength demonstrated in a text, which is used in false-news detection approaches as a system or complementary component [3]. It can be as easier as these binary positions such as positive and negative or sometimes the classification will be neutral. Sentiment analysis from text is beyond polarization and may include the determination of users' emotional conditions such as depression, anxiety,

excitement and anger [11]. Some sense dictionaries can help accomplish this task. Sentiment analysis from text like blogs, Twitter and news channels are fine-researched topic fields. However, this is the initial time research has been managed in the context of identifying false news on online social networks. For the motive of this current work, the perceptual analysis of the text is restricted from text messages to the negative and positive polarities of keywords.

While the above systems take various methods and perspectives, this paper is confined to dealing with fake news in both Machine Learning and NLP including missing data. In-depth, research concerning fake news requires additional statistical techniques to address their spontaneous and unstructured nature for data analysis. One challenge in analysing fake news is to properly handle missing data. As fake news is not created for research, most information within social media and news is not structured in a pre-organized manner. Accordingly, missing values are likely to occur, which leads to inconsistent or biased statistical results when applying regression-based analysis or classification [13, 20, 25]. These missing data problems are often encountered even when dealing with online data in a structured form. Two approaches are primarily used to handle incomplete data with missing values. A naïve approach is a deletion under which observations with missing values are excluded from the data analysis. The other approach is to implement an imputation that replaces missing values with plausible alternative values. Xin Liao studied the data hiding in encrypted images by using CS and discrete fourier transform. This show that this method can reduce the average extracted-bit error rate when the block size is appropriate. In practice, imputation methods are preferred because deletion is inefficient and may cause large biases in the model parameter estimates [19]. Among imputation approaches, multiple imputations, initially proposed by Enders et al. [9], are the most popular in both social science and biomedical science due to their statistical and practical advantages.

Multiple imputations yield precise estimates and accurate standard errors that can help obtain less biased results than when using single imputations [12]. These advantages make multiple imputations one of the best options for handling missing values [22]. As many statistical packages have already been developed for implementing multiple imputations on incomplete data, non-statisticians can easily handle missing values and then conduct statistical analyses on the imputed datasets. Given that online data are likely to have a considerable portion of missing values and be able to provide different forms of auxiliary information useful for creating alternative imputed values, multiple imputations are of great importance for fake news research.

It is of paramount importance that the researchers confront and try to eliminate the problem of fake news. There have been many methods that are proposed ranging from NLP analysis to clustering. Amer et al. [4] applied two machine learning supervised algorithms, i.e., Random forest and decision tree classifiers to detect Coronavirus covid-19 fake news, with this model, Count Vectorizer and Document Frequency Vectorizer as feature extraction after making a set of the initial set such as pre-process and normalization of the dataset. However, this work was presented during the labelling of the posts, demanding a lot of exhausting time in addition to labelling, and keeping up to date with what was happening during the pandemic. With that, the labels themselves were chosen, which may have occurred some mistakes during labelling.

Zhou et al. [28] proposed a theory-driven model for fake news detection. Fake news detection is then conducted within a supervised machine learning framework which enhances the interpretability of fake news feature engineering, and studies the relationships among fake news, deception/disinformation, and click baits. Experiments conducted on two real-world datasets indicate the proposed method can outperform the state-of-the-art and enable fake news early detection when there is limited content information. Datasets consisting of the ground truth of, e.g., both fake news and clickbait, are invaluable to understanding the relationships

among different types of unreliable information; however, such datasets are so far rarely available. Furthermore, it should be pointed out that effective utilization of rhetorical relationships and utilizing news images in an explainable way for fake news detection are still open issues. Kaliyar et al. [17] proposed coupled matrix–tensor factorization method to get a latent representation of both news content as well as social context. To classify news content and social context-based information individually as well as in combination, a deep neural network was employed with optimal hyper-parameters. For the task of fake news detection, a feature set can never be considered complete and sound. Jiang et al. [15] evaluated the performance of five machine learning models and three deep learning models on two fake and real news datasets of different sizes withholding out cross-validation. Moreover, the detection of fake news with sentiment analysis is required for different machine learning and deep learning models.

To fill this research gap and with the discussions, this study aims to introduce a new multiple imputation method for fake news detection research that incorporates social media and news content including both structured opinions shared via sub-ratings like user opinions in comments on specific content attributes and unstructured opinions in the form of text. This imputation model also adopts sentiment analysis. In terms of English word segmentation, since traditional machine learning methods cannot solve the long-distance dependencies of texts, it is difficult to analyse the information contained in the problem as a whole and grasp the user's true intention. Therefore, different machine learning-based models are implemented to detect and classify fake news. Each model's performance is measured to categorize various news items correctly, which revealed each model's ability to improve its accuracy in detecting fake news.

The rest of this paper is organised as follows, section 2 contributes a literature survey with different existing research. Section 3 studies the proposed methods carried out in this study. Section 4 analyse the implementation of the study. Finally, the study concludes with concepts in section 5.

2 Literature survey

Disseminated information and its dissemination process build a major problem in detecting these contents immediately, thus highlighting the importance of automatically identifying false news. To overcome this, Sahoo et al [23] proposed an automatic fake news identification technique for the environment chrome using this the detection of fake news on Facebook is possible. Specifically, this uses multiple features associated with a Facebook account in addition to some news content features to analyse the characteristics of the account across deep learning. Shu et al [24] present FakeNewsNet, a repository of fake news data, the news content includes two complete datasets with different features, spatiotemporal information, and social context to make facilitate fake news-related research. This comprehensive description of FakeNewsNet displays an analytical analysis of two datasets from various viewpoints and discussed the advantages of FakeNewsNet for potential applications in social media fake news research. SAF/S performs better in terms of accuracy and F1 score. SAF/A provides a similar result with 66.7% accuracy as SAF/S. This indicates that user engagements can help fake news detection in addition to news articles on the PolitiFact data set. Meanwhile, the selection strategy can be used for web search results to reduce noise in the data collection process.

The studies on user credibility in this context focus more on the frequency and timing of engaging in fake news propagation, rather than specification according to the content of users' tweets. Duan et al [8] approach this challenge by elaborating two features one is linguistic and another one is a sentiment feature from operators' tweet feed as well as retrieving the presence of hashtags, emojis, and political bias in their tweets. These features were later used to categorize operators as those who broadcast or did not broadcast fake news. 72% accuracy was obtained by this proposed approach, among the results in the first 4 positions acquired by systems for the task in the English language. Yet, in applications with diverse classification algorithms and the union of the different representations, not all combinations of representations increased the accuracy. NER in combination with other representations is not suitable for the use of SVMs or ANNs. Moreover, this limit had to be raised multiple times. The need for this is probably due to a large number of features (416,834).

A domain reputation analysis was proposed by Xu et al [26] that reveals the internet pages of real and fake news publishers revealing different registration behaviours, registration time, domain rankings and domain popularity. In addition, fake messages will disappear from the Internet after a certain time. This content on the false and original news corpus is unskilful in detecting false news, using time frequency-inverse document frequency (tf-IDF) and Latent Dichotomy Allocation (LDA) header modelling, while exploring document compatibility with word and word. Vectors are the most promising direction to predict original and false news. This shows the promising aspect of leveraging document similarity to distinguish fake and real news by measuring the document similarity of the news under tests with the known fake and real news corpus. On the other hand, the difference in the topics and word embeddings shows little or subtle difference between fake and real news.

Kumar et al [18] proposed a CNN + bidirectional LSTM ensembled network to gather fresh instances such as PolitiFact and build multiple information for the identification of original and false news and match multiple state-of-the-art approaches. Long Short-Term Memories (LSTMs), Convolutional neural networks (CNNs), attention mechanisms and ensemble methods are examples of multiple state-of-the-art approaches. This research collects 1356 news instances from various users via Twitter and media sources such as PolitiFact and creates several datasets for the real and the fake news stories. The study conclude that CNN + bidirectional LSTM ensembled network with attention mechanism achieved the highest accuracy of 88.78%, whereas Ko et al. tackled the fake news identification problem and achieved a detection rate of 85%. As the result, CNN + bidirectional LSTM ensembled network with focus mechanism obtained 88.78% of maximum accuracy. The results were satisfactory but not promising. The CNN architecture gave the lowest accuracy in comparison to the others that we studied. The LSTM architecture and bidirectional LSTM architecture performed significantly better in comparison to simple CNN architecture. We further increased our appetite for improved accuracy and incorporated more complex models as part of our methodology.

The hybrid deep learning design that merges recurrent neural and convolutional networks for false news identification was proposed by Nasir et al [21]. On two fake news datasets (ISO and FA-KES) this model was certified successfully, achieving the results of detection that are substantially better than other non-hybrid foundation techniques. A paired t-test was used to validate the statistical significance of the results; the experiments were repeated five times (using 5-fold cross-validation, i.e. 80%–20% split); and accuracy was reported at 95% confidence intervals. ISOT is chosen for training because it is much larger and has minimum space for improvement since many models perform above the 0.9 classification accuracy

threshold. Moreover, complex neural network architectures not be considered as part of the study.

A deep convolutional neural network (FNDNet) for false news detection was proposed by Kaliyar et al [16]. This prototype (FNDNet) is outlined instead of relying on hand-crafted features to learn automatically, about the one-sided features for false news identification build in the deep neural network across many hidden layers. As the result, each layer contains many features that will be extracted by a deep Convolutional Neural Network (CNN). Benchmarked datasets were used to train and test the model, and the proposed model achieved state-of-the-art results with an accuracy of 98.36% on the test data. Various performance evaluation parameters such as Wilcoxon, false positive, true negative, precision, recall, F1, accuracy, etc. were used to validate the results. Despite the high performance of our classifier, there is a scope for improvement. A multi-model approach (a combination of different learning techniques) is the main necessity for fake news detection for solving the multi-class fake news detection problem.

Choudhari et al [6] proposed a linguistic model to identify the properties of the content and language-driven features will also generate with the help of this. This linguistic prototype extracts particular news features such as syntactic, sentimental, grammatical, and readability. The language-driven model demands an approach to managing handcrafted feature problems and is time-consuming to maintain the trouble of dimensionality problems. Therefore, a continuous learning model based on neutrality is utilized to achieve the best results for detecting fake news. The results are drawn up to verify the importance of the extracted features of the linguistic model and finally, the integrated linguistic feature-driven model that can achieve an average of 86% accuracy in detecting and categorizing fake messages. However, extensive features/parameters for model performance are lacking. Examine the latent semantic feature-driven fake news detection model, and explore various variants of convolution neural networks for image-driven fake news detection.

The Structure-aware Multi-Head Attention Network (SMAN) was proposed by Yuan et al [27], which merges the content of news, issuing, and reposting connections of users and publishers, to collectively optimize the credibility prediction tasks and fake news detection. As a result, we can explicitly make use of publishers' and users' credibility to detect early fake news. The research conducted experiments on three real-world datasets, and the results show that SMAN can detect fake news in 4 hours with an accuracy of over 91%, which is much faster than the state-of-the-art models.

Dang et al [7] utilize Term Frequency-Inverse Document Frequency (TF-IDF) and word embedding has been implemented to the whole range of datasets to solve the problems in sentiment analysis, for example, sentiment polarity. As a result, comparative knowledge was carried out on the experimental outputs obtained for various designs and input features. The experiments also revealed that CNN outperforms other models, presenting a good balance between accuracy and CPU runtime. RNN reliability is slightly higher than CNN reliability with most datasets but its computational time is much longer. However, exploring hybrid approaches, where multiple models and techniques are combined to enhance the sentiment classification accuracy achieved by the individual models or techniques, as well as to reduce the computational cost is the reliable cost.

Abdullah et al [14] used the multimodal approach with Convolutional Neural Network (CNN) and Long Short-Term memory (LSTM) to classify the fake news articles that achieved significant performance. We worked on a database with 12 different categories of news articles and used linguistic cue approaches with machine learning. We classified news based on its

source and its previous history (such as domain name and/ or author name) with bimodal CNN and LSTM. Through reputable news sources, the model classifies reliable news articles with an accuracy of 99.7% on the training data and 97.5% on test data. However, as a piece of fake news can still be published on a reputable domain, we still had to consider other parameters such as news headlines.

Nida Aslam et al [5] proposed an ensemble-based deep learning model to classify news as fake or real using a LIAR dataset. Due to the nature of the dataset attributes, two deep-learning models were used. For the textual attribute "statement," Bi-LSTM-GRU-dense deep learning model was used, while for the remaining attributes, the dense deep learning model was used. Experimental results showed that the proposed study achieved an accuracy of 0.898, a recall of 0.916, a precision of 0.913, and an F-score of 0.914, respectively, using only statement attributes. Moreover, the outcome of the proposed models is remarkable when compared with that of the previous studies for fake news detection using the LIAR dataset. Despite the significant results achieved by the proposed study, there is still room for improvement. The model needs to be investigated using other fake news datasets.

From the aforementioned studies, the research gap present in each manuscript motivates me to study the hybrid methods for fake news detection. Among the various hybrid methods that exist in the literature, those that model the social graph that spreads the news, or the user and news source features (profile), cannot be applied when only the text of the news is available. From the hybrid methods that examine only the textual content of news, the combination of LSTM and CNN has shown promising results. However, so far, LSTMs have been used for providing word embeddings and CNN for doing the final classification. Accordingly, the research proposed Naïve Bayes, a passive-aggressive classifier and Deep Neural Network (DNN) to be implemented to detect fake news with multivariate missing values to tackle the issue [16]. The passive-aggressive classifier is used for training the ISOT and LIAR dataset and the Naïve Bayes was used to test the model for detecting fake news. Finally, DNN is used for validation purposes which efficiently classify fake and real news. A conclusion to the analysis of the related literature is that fake news has played a significant role in many real-time disasters. In order, manual interventions are of no use due to the multiple datasets which contain information sharing on the internet. Machine learning techniques have experimented on a range of datasets and deep learning techniques are still to be fully evaluated on fake news detection and related tasks. Table 1 illustrates the comparison of the state-of-the-art techniques.

3 Research proposed methodology

The usage of social media platforms has been increasing day by day. Due to the absence of regular supervision and oversight, and by lack of accountability, violators have been able to run uncontrolled and propagate false information. Therefore, the detection of fake news from social media is important for the current situation. Consequently, the article proposed novel fake news detection based on multivariate missing variables with useful features using classifier techniques.

The architecture of the proposed methodology is illustrated in Fig. 1. Intially, the latent variable formation is handled by key feature words with the content's propensity scores of the opinions on sentiment analysis using a lexicon-based scoring algorithm. Further, the paper, proposed a new multiple imputation strategy for handling multivariate missing variables in the ISOT of social media data. The researchers have used this imputation approach to bring in

Table 1 Comparison table for the state of art techniques

References	Objective	Pros	Cons
Sahoo et al [15]	Automatic fake news detection approach in chrome environment using machine learning and deep learning classifiers on which it can detect fake news on Facebook.	This analyzes both user profile and news content features	
Shu et al [23]	Fake news data repository FakeNewsNet contains two comprehensive data sets with diverse features in news content, social context, and spatiotemporal information.	FakeNewsNet would benefit the research community by studying various topics such as (early) fake news detection, fake news evolution, fake news mitigation, and malicious account detection.	This only shows the metadata of 5000 users in the provided link due to the space limitation.
Duan et al [24]	Multiple machine learning and deep learning algorithms to obtain the highest accuracy for detecting fake news patterns	Testing set on TIRA showed 70% accuracy on our highest-achieving model	Not all combinations of representations increased the accuracy. NER in combination with other representations is not suitable for the use of SVMs or ANNs.
Xu et al [8]	Term frequency-inverse document frequency (tf-IDF) and Latent Dirichlet Allocation (LDA) topic modelling is inefficient in detecting fake news,	Domain reputations and content characteristics of fake and real news will provide key insights for effectively detecting fake news on social media.	The difference in the topics and word embeddings shows little or subtle difference between fake and real news
Kumar et al [26]	Compare the CNN, LSTM, bidirectional LSTM model, CNN+LSTM ensembled network, and bidirectional LSTM+LSTM ensembles model to gather fresh instances such as PolitiFact and build multiple information for the identification of real and fake news	Use this research to combat fake news stories and neutralize the drastic effects of false information on a large scale	This research focussed mostly on the sentiments of news stories while not paying continuous attention To the credibility of the news sources themselves, due to resource limitations. Second, the classification models were unable to identify the semantic transition of real news to fake news
Nasir et al [18]	Proposes a novel hybrid deep learning model that combines convolutional and recurrent neural networks for fake news classification.	Approximately 100% accuracy on the ISOT dataset,	Over-fitted models expose high complexity and examine a lot more information than is probably needed to reach a decision.
Kaliyar et al. [21]	Deep convolutional neural network (FNDNet) for fake news detection.	This research will assist researchers in broadening the understanding of the applicability of CNN-based deep models	A hybrid approach can create more impact in the case of multi-label datasets.
Choudhari et al [16]	The linguistic model with neural-based sequential learning is proposed for fake news detection	Measure the importance of extracted feature sets as well and readability is considered the most rarely used feature out of all extracted feature	Extensive features/parameters for model performance are lacking

Table 1 (continued)

References	Objective	Pros	Cons
Yuan et al [6]	Structure-aware Multi-head Attention Network (SMAN) for fake news detection	SMAN can not only improve the detection performance but also significantly reduce the time required for the detection	–
Dang et al [27]	Deep learning models and related techniques applied to sentiment analysis for social network data	Good balance between accuracy and CPU runtime.	Address the problem of aspect sentiment analysis
Abdullah et al [7]	Multimodal approach with Convolutional Neural Network (CNN) and Long Short-Term memory (LSTM) to classify the fake news articles	The model classifies reliable news articles with an accuracy of 99.7% on the training data and 97.5% on test data	Fake news can still be published on a reputable domain, we still had to consider other parameters such as news headlines.
Nida Aslam et al [14]	Ensemble-based deep learning model to classify news as fake or real.	The outcome of the proposed models is remarkable when compared with that of the previous studies	Multiple dataset analysis is necessary.

missing values of sub-ratings on content posts in social media and the news. This suggested technique generates multiple imputed values for missing text content values based on sentiment analysis of text contents and verified user opinions. When compared to deletion and other imputation strategies, this imputation method is used to reduce inaccuracy and bias in the results of the study on online data. Subsequently, the useful features have to be extracted from the social media or news contents using Term Frequency And Inverse Document Frequency (TF-IDF). Finally, the classification of the detection of fake news has to be made, which has been done using multiple techniques, such as Naïve Bayes, passive-aggressive classifier, and Deep Neural Network (DNN). The description of the proposed method is detailed in further sections.

3.1 Data collection

For the identification of false news, numerous datasets have been provided. Having a big dataset for training the model is among the most important prerequisites for employing neural networks. Researchers employ two datasets for training deep models in this paper: ISOT false news and LIAR, both of which include a significant amount of documents. The size of ISOT's news statements is medium to long, whereas LIAR's is modest. There is both actual and fake news in the databases. The real news was gathered via reading articles from Reuters.com, while false news was gathered from untrustworthy sources highlighted by Politifact; within that case, the researchers additionally used data from FakeNewsNet.

3.2 Initial structure

Let X be the overall user opinions score, Y_k be the kth attribute user opinions score, $k = 1, \ldots, K$ and W be the user opinions. Assume that X and W are observed over the entire data set but Y_k are subject to missingness. Now let R_k denote a set of indicators of the missingness Y_k. R_k takes

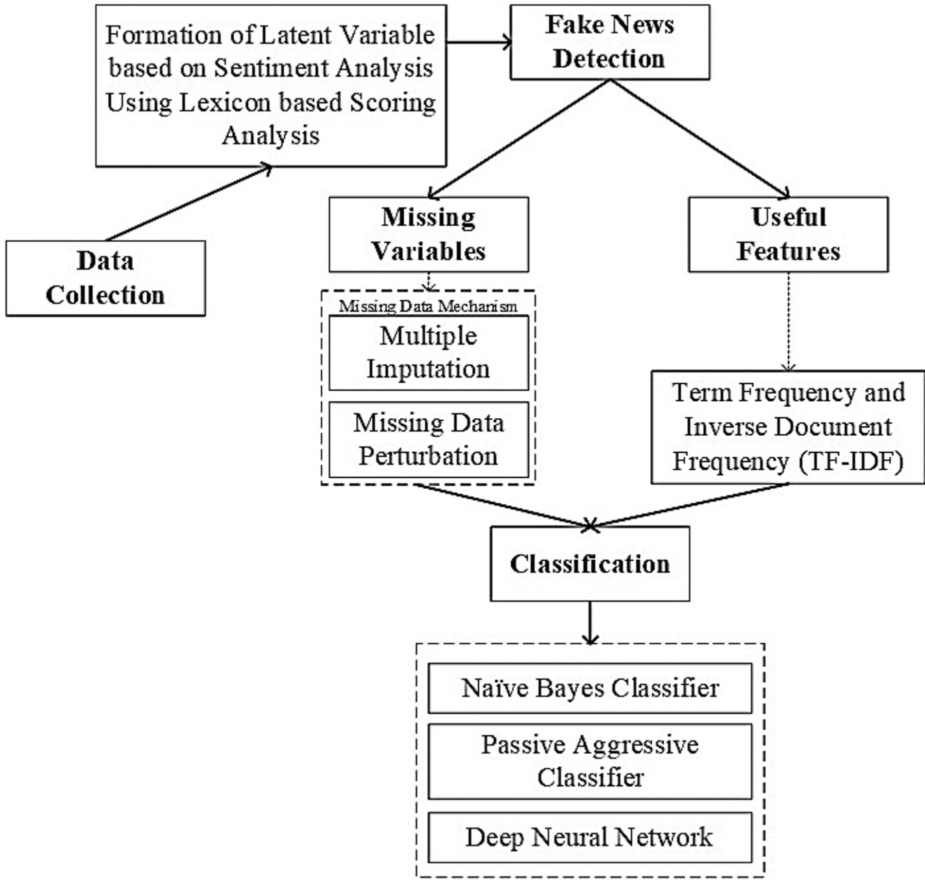


Fig. 1 Architecture of the proposed method

the value of 1 if Y_k is observed and 0 otherwise. Let Y_{obs} and Y_{mis} be the observed/ missing values of $Y = (Y_1\ldots,\ Y_k)$.

Assume MAR missing mechanism such that:

$$P(R|Y,X,W) = P(R|X,W) \tag{1}$$

Where $R = R_1, \ldots R_k$. Thus, the missing values can be generated without specifying the response model.

3.2.1 Formation of a latent variable Z

Consider the predictive distribution of the Y conditional on X and W as the imputation model:

$$P(R|Y,X) \tag{2}$$

One simple method for generating the imputed values for Y is to use only X because W is unstructured, making it difficult to estimate the predictive distribution Eq. (2). However, this method may cause efficiency loss or biased estimation results when the user opinions are

strongly correlated with W given X. To avoid these problems, it needs to consider W, which makes fuller use of the observed text data.

For this purpose, this study needs to structure the unstructured W by creating a latent variable Z that can substitute W. This property of W is formulated as follows:

$$P(Y,X|W,Z) = P(Y,X|Z) \tag{3}$$

where $Z = g(W)$ is a numerical variable constructed from W through a converting mechanism $g(.)$. Text clustering, scoring analysis or similar statistical methods can be applied to obtain Z. Since Eq. (3) is not testable in practice, this study generally assumes that the newly created variable Z from the converting algorithm will be sufficient statistics of W, that is, Eq. (4) is satisfied.

The latent variable Z could be continuous or categorical, but we want this latent variable Z to satisfy the following desirable property:

$$P(Y,X,R|Z = z_1) \neq P(Y,X,R|Z = z_2)\ for\ z_1 \neq z_2 \tag{4}$$

Equation (3) implies that the latent variable Z is informative in explaining the joint distribution of (Y, X, and R). Equation (4) can be decomposed into two properties as follows:

$$P(Y,X|Z = z_1) \neq P(Y,X|Z = z_2)\ for\ z_1 \neq z_2 \tag{5}$$

$$P(R|X,Z = z_1) \neq P(R|X,Z = z_2)\ for\ z_1 \neq z_2 \tag{6}$$

If at least one of two properties is satisfied, we can conclude that Z satisfies Eq. (4). If Z fails to meet Eq. (5) or Eq. (6), the imputation estimates may have larger standard errors than the estimates obtained using a simpler model $P(Y| X)$. On the other hand, if Z satisfies one of two conditions, adding an extraneous variable will not lead to additional biases, and the predictive distribution Eq. (3) will provide more robust results compared to when using a simpler model $P(Y| X)$, which is possibly exposed to the omitted variable problem. Thus, it is important to check if the created variable Z follows the required Eq. (5) or Eq. (6) to validate the new imputation method.

3.2.2 Lexicon-based scoring analysis

This study proposes a new method that does not require any training procedure or similarity score computation to construct a latent variable Z for our imputation. First, the proposed method chooses key feature words based on the parts of speech (POS) in the text content and then compute users' propensity scores of the opinions based on sentiment analysis. The proposed method essentially uses a lexicon-based scoring algorithm. As the false text content with users' subjective opinions is well represented through specific POS such as an adjective, adverb, and verb, this study extracts these POS and then makes scores on these selected features. The detailed algorithm is given below:

Step 1: Assign POS on words within each user's opinions and the main text contents using the natural language processing (NLP) toolkit.

Step 2: Select key feature words categorized as verbs, adverbs and adjectives.

Step 3: Assign one of three values {positive; neutral; negative} to each keyword based on a dictionary of sentiment words, called a sentiment lexicon.

Step 4: Compute a propensity score for user opinions i:

$$S_i = \left(C_{pos,i} + C_{neg,I}\right)/(\alpha + C_i) \tag{7}$$

In the realm of fake news detection, we regard each word feature as a treatment and each news sample as a subject. Then we formally define the propensity score as above. Where, ($\alpha > 0$) is a tuning parameter, C_i is the number of key feature words and $C_{pos,\,i}$ and $C_{neg,\,I}$is the number of positive/negative key feature words. The tuning parameter in step 4 adjusts the normalized degree. For short texts, the propensity scores will be more easily influenced by the sentiment of one key feature word than those of long texts. Thus, this tuning parameter is used to adjust the balance between short and long text data.

This procedure of constructing Z requires text-mining knowledge. There are many packages in Python that provides the proposed scoring algorithm. The NLP toolkit can be used to determine a POS for each word in text data.

Grammatical evidence To inspect real news and fake news, the grammatical feature is an important factor that is extracted through parts of speech (POS) tag evidence features. Out of all POS features, for the targeted problem noun, verbs, adjectives, and pronouns are viable features to define their authenticity. These features are designed to apprehend the deceiver cues in writing style to differentiate fake news. Details are shown in Table 2.

For a given news, defining the grammatical features references as noun count (x_{nou}), verb count (x_{verb}), adjective count (x_{adj}), adverb count (x_{adv})and pronoun count (x_{pro}).

$$X_{gr} = \left\{x_{nou}, x_{verb}, x_{adj}, x_{adv}, x_{pro}\right\}$$

3.3 Missing variables

Social media has become a widespread element of people's everyday life, which is used to communicate and generate content. Among the several ways to express a reaction to social media content, "Likes" are critical. Indeed, they convey preferences, which drive existing markets or allow the creation of new ones. Nevertheless, the situation does not allow to give a dimension to the target universe of the respondents, leading to caution in the management of the missing values. Regarding the statistical analysis, the treatment of missing data represents a relevant problem.

Table 2 Grammatical-based features

Grammatical based Features	Example	Representation
Noun Verb adjective adverb verb	Refugees can be singled out for a higher level of review based on their age, nationality, or gender.	Refugees Higher level gender Singled out

3.3.1 Missing data mechanism

This study develops a new multiple imputation method that can handle multivariate missing variables in social media or news data. Specifically, this imputation method is applied to missing values of user opinions on the posts or the news updates in social media texts. Based on the sentiment analysis of social media or news text data and observed user opinions of likes and comments, this method creates multiply imputed values for missing values of the post or news contents and the user opinions. It is important to first distinguish between missing data patterns, which describe observed and missing values, and missing data mechanisms, which relate to the probability of missingness. Common missing data patterns in surveys typically include unit nonresponse, where a subset of participants do not complete the survey, and item nonresponse, where missing values are concentrated on particular questions. In opinion polls, nonresponse may reflect either refusal to reveal a preference or lack of a preference.

Let $Y = y_{ij}$ be a $(n \times K)$ dataset with each row $y_i = (y_{i1}, \ldots, y_{ik})$ the set of y_{ij} values of feature Y_j for example i. Let Y_{obs} define the observed values of Y and Y_{mis} define missing values. Let M define the missing data identity matrix $M = m_{ij}$, where $m_{ij} = 1$, if y_{ij} is missing and $m_{ij} = 0$, if y_{ij} is nonmissing. The missing data mechanism is missing completely at random (MCAR) if the probability of missingness is independent of the data, or

$$f(M|Y,\varphi) = f(M|\varphi)\ \forall Y,\varphi \tag{8}$$

where φ denotes unknown parameters. The MAR assumption is less restrictive than MCAR in that the probability of missingness depends only on the observed data, $f(M|\ Y,\ \varphi) = f(M|\ Y_{obs},\ \varphi)$ for all Y_{mis}, φ. The missing not at random (MNAR) assumption is that the probability of missingness may also depend on the unobserved data, $f(M|\ Y,\ \varphi) = f(M|\ Y_{obs},\ \varphi)$ for all Y_{mis}, φ. Researchers typically assume data are missing at random (MAR), which mitigates the identifiability problems of MNAR because the probability of missingness depends on data that are observed on all individuals. It is important to grasp these different types of missing data from a statistics point of view. The type of missing data will influence the thanks to accommodate filling within the missing values and detect missing values, and do some basic imputation and detailed statistical approach for handling missing data. Before, joint into code, it’s important to grasp the sources of missing data. To investigate the multi-variate processes, data cleaning/preparation includes renaming the provided dataset and deleting columns, among other things. Based on Eq. (3), this study uses the widely used Multiple Imputation Chain Equation (MICE) for multiple imputations. The methods and techniques for cleaning data will differ depending on the dataset. The fundamental purpose of data cleaning is to find and eliminate mistakes and abnormalities to improve the quality of the dataset. The primary goal of data cleaning is to detect and remove errors and anomalies to increase the price of data in analytics and better noesis.

3.3.2 Multiple imputation

A modified imputation procedure is summarized below:

Step 1: Convert W to a latent variable Z using sentiment analysis on text data.
Step 2: Specify the imputation model for each missing variable with the fully observed data:

$$P\left(Y_k|Y_{-k},X,Z,\delta=1;\theta_k^{*(0)}\right),\ for\ k=1,\ldots,K \tag{9}$$

where Y_k denotes the collection of Y except Y_k, $\delta=\Pi_{k-1}^{K}R_k$, and $\widehat{\theta}_k^{*(0)}$ denotes the parameter estimates of the imputation model for the kth missing variable on the complete cases.

Step 3: For given imputed values $Y^{*(t-1)}=\left(Y_{obs},Y_{mis}^{*(t-1)}\right)$, the ith iteration of MICE is a Gibbs sampler that sequentially generates:

$$\widehat{\theta}_1^{(t)}\sim P\left(\theta_1|Y^{*(t-1)},X,Z\right) \tag{10}$$

$$Y_1^{*(t)}\sim P\left(Y_1|Y_{-1}^{*(t-1)},X,Z,\widehat{\theta}_1^{(t)}\right) \tag{11}$$

$$\widehat{\theta}_2^{(t)}\sim P\left(\theta_2|Y_1^{*(t)},Y_{-1}^{*(t-1)}X,Z\right) \tag{12}$$

$$Y_2^{*(t)}\sim P\left(Y_2|Y_1^{*(t)},Y_3^{*(t-1)},\ldots,Y_k^{*(t-1)}X,Z,\widehat{\theta}_2^{(t)}\right) \tag{13}$$

$$\widehat{\theta}_K^{(t)}\sim P\left(\theta_K|Y_{-K}^{*(t)},Y_K^{*(t-1)}X,Z\right) \tag{14}$$

$$Y_K^{*(t)}\sim P\left(Y_K|Y_{-K}^{*(t)},X,Z,\widehat{\theta}_K^{(t)}\right) \tag{15}$$

Step 4 Iterate step 3 for large enough t until we have convergence.
Step 5 Independently repeat steps 3 and step 4 M(>1) times so that this research creates M imputed data sets as the final imputation output.

Consider an imputation estimator constructed from the multiply competed for datasets. Let Q be an estimand defined with a known link function h, where $Q = h(Y, X)$. On the multiply completed data sets, the imputation estimator of Q is:

$$Q=M^{-1}\sum_{m-1}^{M}\widehat{Q}_m=M^{-1}\sum_{m-1}^{M}h\left(Y_m^*,X\right) \tag{16}$$

where Y_m^* denotes a set of imputed values of the *m*th completed data set, and $\widehat{Q}_m = h\left(Y_m^*, X\right)$ is the estimates obtained from the *m*th completed data set. The variance of the imputation estimator can be estimated using the variance formula, where:

$$\widehat{V}\left(\widehat{Q}\right) = \widehat{A} + \frac{M+1}{M}\widehat{B} \tag{17}$$

With the average of within-imputation variances:

$$\widehat{A} = M^{-1}\sum_{m-1}^{M}\widehat{V}\left(\widehat{Q}_m\right) \tag{18}$$

and the between-imputation variance:

$$\widehat{B} = (M-1)^{-1}\sum_{m-1}^{M}\left(\widehat{Q}_m - \widehat{Q}\right)\left(\widehat{Q}_m - \widehat{Q}\right)^T \tag{19}$$

3.3.3 Missing data perturbation

After randomly splitting each dataset, this study perturbs the training data so that the proportion of missing values in the set of categorical features Y_{cat} follows $\delta = \{0.1,\ 0.2,\ 0.3,\ 0.4\}$ according to the MCAR mechanism

$$\Pr(M = 1 | Y_{cat} \cdot \varphi) = \delta \ for\ all\ Y_{cat} \tag{20}$$

This research uses missing-data perturbation to study the impact of larger amounts of missing data; however, it is also a form of dropout noise that can be used to control overfitting during the training process and improve the generalizability of the model.

3.4 Useful feature extraction

Feature extraction solves the problem of finding the most compact and informative feature set. For classification and regression problems, defining feature vectors remains the most common and convenient method of data representation. The commonly used feature extraction method is driven by the size of the data table. With the increasing efficiency of data storage, the size of data tables is also increasing. Extracting effective features from text and avoiding useless data processing is the key to experimental research.

There are various "sizes" and "forms" of data in text information. An important point when extracting features in the text is structured data. Generally, the raw data that has not been processed in the text is converted into structured data. The process of tools to get valid information is called information extraction. In the same way, in this research, extracting useful features from the actual news content is a challenging task because fake news spreaders could make the content of the fake news look like real news.

3.4.1 Term frequency and inverse document frequency (TF-IDF)

In this work, the proposed method used term frequency and inverse document frequency (TF-IDF) to identify the useful features of news content. Existing work on fake news detection can be divided into two categories: unimodal and multimodal. To learn a more general joint

representation, a minimax game is set up between the event discriminator and feature extractors. Scaled-dot product attention has been applied to the fields of natural language processing (NLP) and computer vision (CV). In NLP and CV, the extraction of corresponding features, such as textual features and visual features, is a fundamental task, and it is also a key step in fake news detection. In this section, we review the related work on unimodal fake news detection, and hybrid fake news detection with a useful feature extraction technique is introduced. Term frequency works by looking at the frequency of a particular term you are concerned with relative to the document. Inverse document frequency looks at how common (or uncommon) a word is amongst the corpus. To summarize the key intuition motivating TF-IDF is the importance of a term is inversely related to its frequency across documents.TF gives information on how often a term appears in a document and IDF gives information about the relative rarity of a term in the collection of documents. TF-IDF technique is used to produce a composite weight for each term in the document which is called *tf-idf* weight. Calculating *tf-idf* weight has great importance in information retrieval and text mining tasks as it determines the significance of a term or word in a document as well as in a corpus.

$$tf{-}idf_{t,d} = tf_{t,d} \times idf_t \tag{21}$$

In Eq. 21, t means a term and d refers to a document. The term frequency $f_{t,\ d}$ means the measure of the frequency for a particular term t in a document, in other words, how many times term t appeared divided by the total number of terms in the document and inverse document frequency idf_t is the logarithm of a total number of documents in the corpus divided by the number of documents where term t appears. idf_t the measure helps in knowing the importance of the term t. From this method, this study observed that the derived weight matrix represents a large number of term features.

3.5 Classification

This study has thoroughly used ML to build the proposed models. The task of choosing the classifiers that emerged from the suitable properties of algorithms is, therefore, developed as a hybrid classifier model. As Naïve Bayes is popular for its multi-class prediction, it was picked up for its ease and robustness in predicting the class of the text. One of the problems with other methods is when new samples are collected, a model must be retrained to predict the output for new data. This is overcome by using a passive-aggressive classifier which trains the model incrementally, allowing modifications of the parameters only when needed, while discarding these updates when they don't alter the equilibrium. This study focused on the problem based on both conventional and deep learning architecture. A deep neural network was used to increase the efficiency in the identification of fake news. The following paragraphs dive deeper into each algorithm.

3.5.1 Naïve Bayes classifier

A naïve Bayes classifier assumes that features are statistically independent of one another. It explicitly models the features as conditionally independent given the class. Because of the independence assumption they are highly scalable and can quickly learn to use high-dimensional features with limited training data. Given a data point $\overrightarrow{x}$ of n features, Naïve Bayes predicts the class C_k for a data point, according to Bayes' theorem it can be factored as,

$$p\left(C_j|\vec{x}\right) = \frac{p\left(\vec{x}|C_j\right)p\left(C_j\right)}{p\left(\vec{x}\right)} = \frac{p\left(x_1,\ldots,x_n|C_j\right)p\left(C_j\right)}{p\left(x_1,\ldots,x_n\right)} \tag{22}$$

If this study uses the simplifying conditional independence assumption, that given a class (positive or negative), the words are conditionally independent of each other. Due to this simplifying assumption, the model is termed “naïve”.

$$p\left(C_j|\vec{x}\right) = \frac{\left(\Pi p\left(x_i|C_j\right)\right)p\left(C_j\right)}{p\left(\vec{x}\right)} \tag{23}$$

Here the x_i represents the individual words of the document. The classifier outputs the class with the maximum posterior probability.

Laplacian smoothing If the classifier encounters a word that has not been seen in the training set, the probability of both the classes would become zero and there won’t be anything to compare between. This problem can be solved by Laplacian smoothing.

$$p\left(x_i|C_j\right) = \frac{Count\ (x_i) + k}{(k+1)\left(No\ ofwordsinclass\ C_j\right)} \tag{24}$$

Usually, k is chosen as 1. This way, there is an equal probability for the new word to be in either class. Since Bernoulli’s Naïve Bayes is used, the total number of words in a class is computed differently. For this calculation, each document is reduced to a set of unique words with no duplicates.

As considered, this classifier is best suited for small-size datasets, this study proposed a passive-aggressive classifier due to its specific properties.

3.5.2 Passive aggressive classifier

A passive-aggressive classifier is simple and its performance has been proven to be superior to many other alternatives. Let’s suppose to have a dataset where $\overline{x}$ is the datapoint and y_i is the predicted output. Given a weight vector w, the prediction is simply obtained as: $\widetilde{y}_t = sign\left(\overline{w}^T \cdot \overline{x}_t\right)$, the algorithm works generically with this update rule:

$$\overline{w}_{t+1} = \overline{w}_t + \frac{\max\left(0, 1 - y_t\left(w^T.x_t\right)\right)}{\|x_t\|^2 + {}^1/_{2C}} y_t\overline{x}_t \tag{25}$$

Hence this classifier trains the model incrementally. These are the conventional means of algorithms whose accuracy is limited when compared with deep learning architecture.

3.5.3 Deep neural network

The researchers chose to use tokenizers for feeding into Deep Neural Network models. Python offers a simple API, where one can vectorise text corpus by converting each word into vectors or sequences of integers. It splits the text into a list of tokens where the coefficient for each

token could be based on word count. The dictionary of the tokenizer has been prepared using the train set of each dataset. The tokenizer once constructed can be fit on the raw text data. Since each different article contained different number of words, this study used padding to keep the size uniform.

There are mainly 7 layers i.e. 1 input (takes prior model input), 1 output, and 5 hidden layers. Each attribute is individually tokenized and fed to the network. Each input layer takes the tokenized value from a single attribute of the dataset. Each Input layer is fed to the embedding layer which learns all the embeddings of the word. This layer allows us to take large inputs like sparse vectors representing words. The first DNN layer defines a filter of kernel size 3. Now there are 4 × 4 weights assigned to all neurons in the same block. The output of this layer is fed to a global max pool layer which is used to compute the single max value for each of its input channels. It is a great alternative to flattening. All three outputs from the max pool layers are concatenated to form a single layer. This layer is fed to a series of Dense layers which connects all the neurons to form a network. The final output is fed to the output layer, here CNN is connected to fully connected NN for decision making as either true or false. The fully connected layer with sigmoid activation is the final layer that will reduce the vector of height 8 to 1 for prediction ("fake", "real"). The Dense layers contained Relu activation functions. Activity, kernel, and bias regularizers along with Dropout were all used as seen fit.

3.5.4 Overfitting and cross-validation

Overfitting is one of the central problems in machine learning. It arises when the model performs poorly on unseen data while giving excellent results on training data. Cross-validation is a way to overcome such an issue; it aims to test the model's ability to correctly predict new data that was not used in its training. Cross-validation shows the model generalization error and performance on unseen data. K-fold cross-validation is one of the most popular versions. In this experiment, the researchers use k-fold cross-validation to ensure that the study avoids overfitting.

4 Result and discussion

This study employs ISOT and LIAR datasets to verify the proposed models. The dataset contains a total of 44,848 news articles, of which 21,417 are real and 23,481 are fake news. This dataset was applied for the key feature analysis using sentiment analysis and further. Three evaluation metrics, namely accuracy, F1-score and recall are used to evaluate the performance of these proposed models. One of the major challenges of performing classification on this dataset is handling missing values. Therefore, multiple classification techniques were performed to handle and get an accurate result, this classifies the result with the differences between before feature extraction and after feature extraction, and the dataset with imputation and dataset without imputation model is also evaluated.

The experiment of this paper was conducted on a system with a windows 10 operating system, a memory capacity of 6GB DDR3, and an Intel Core i3@ 3.5GHz would be the processor. With this system configuration, the proposed method is implemented using the software of Python.

4.1 ISOT dataset

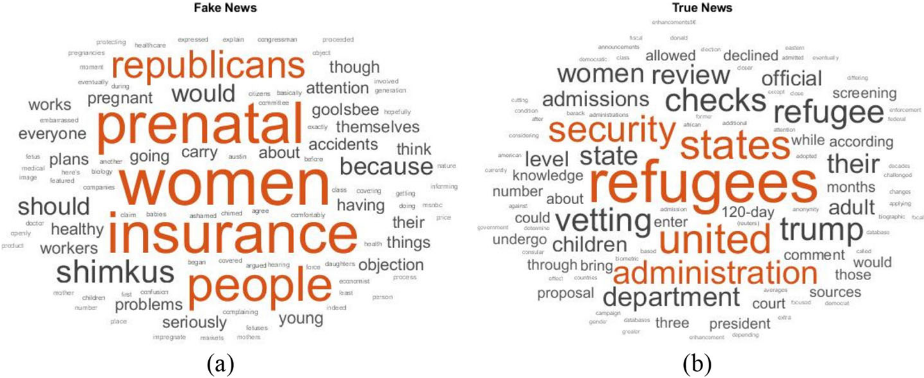
The dataset contains two types of articles fake and real News. This dataset was collected from real-world sources; the truthful articles were obtained by crawling articles from Reuters.com (News website). As for the fake news articles, they were collected from different sources. The fake news articles were collected from unreliable websites that were flagged by Politifact (a fact-checking organization in the USA) and Wikipedia. The dataset contains different types of articles on different topics, however, the majority of articles focus on political and World news topics. The dataset consists of two CSV files. The first file named “True.csv” contains more than 12,600 articles from reuter.com. The second file named “Fake.csv” contains more than 12,600 articles from different fake news outlet resources. Each article contains the following information: article title, text, type and the date the article was published on. The data collected were cleaned and processed, however, the punctuations and mistakes that existed in the fake news were kept in the text.

Figure 2 depicts the ISOT dataset word cloud image of fake and real news. After preprocessing, the number of samples was reduced to 44, 143 (22, 727 fake and 21, 416 real). The similarity between real and fake news post word usage in the ISOT dataset suggests the real and fake news posts are not well separated by word frequencies alone. Most words highlighted in the fake news are prenatal, insurance, Shimkus and republicans and for true words, it seems to be refugees, trump, united, and administration.

4.2 LIAR dataset

LIAR is a publicly available dataset for fake news detection. A decade-long of 12.8 K manually labelled short statements were collected in various contexts from POLITIFACT.COM, which provides a detailed analysis report and links to source documents for each case. This dataset can be used for fact-checking research as well. Notably, this new dataset is an order of magnitude larger than previously largest public fake news datasets of similar type. The LIAR dataset4 includes 12.8 K human-labelled short statements from POLITIFACT.COM’s API, and each statement is evaluated by a POLITIFACT.COM editor for its truthfulness.

To visualize the news content of the dataset, word cloud representations are used. Word cloud representations depict the frequency of the terms in a specific dataset of LIAR. This



(a) (b)

Fig. 2 ISOT dataset Wordcloud of fake and true words

system draws some exciting conclusions from the word cloud representation shown in Figs. 3a, b. Real news word cloud represents important entities that occurred in actual events like the Americans, texas, Obama, and state, while fake news word cloud highlights fake entities, such as Obama, state, president, tax and republican. It was interesting to observe that fake news generally uses appealing headlines and does not have specific content that denotes real news. It is observed that both fake and real news articles are generally related to a political campaign.

4.3 Spam detection with machine learning classifiers

The passive-aggressive classifier was trained on 70% of the ISOT dataset and the Naïve Bayes was used to test the model using the remaining 30% for detecting fake news. Similarly, for the LIAR dataset, 80% was used for training and 20% was used for testing. If the sentiment score is positive, then the sample is tagged as positive for the sentiment attribute, otherwise Negative is assigned. They have achieved good timeefciency performance as well as better classification performance. The model achieves an average accuracy of 90% over a five-fold cross-validation.

Table 3 illustrates the number of attributes in the dataset before and the number of attributes in the dataset after feature extraction for two datasets. The attributes used after feature extraction for the ISOT dataset are title, text subject, date, noun, verb, and adjective. Similarly, for the LIAR dataset, the attributes used after feature extraction are id, title, statement, subject, speaker, speaker job title, state info, party affiliation, noun count, verb count, adjective count and adverb count.

The confusion matrix of the DNN classifier is represented in Fig. 4. True-positive value and true-negative value of the random forest classifier are high means if the news is false, it hows false, and in the case of true news, it predicts the same. Here the output class predicts that fake words of 95% and real words of 67.3% respectively.

4.4 Error rate of dataset with MCAR perturbation

For varying amounts of MCAR-perturbed qualitative training data, error rates on the test set with and without missing data imputation (x-axis). The standard deviation of test set errors of ANNs trained using different convergences is used to calculate prediction intervals for ANNs.

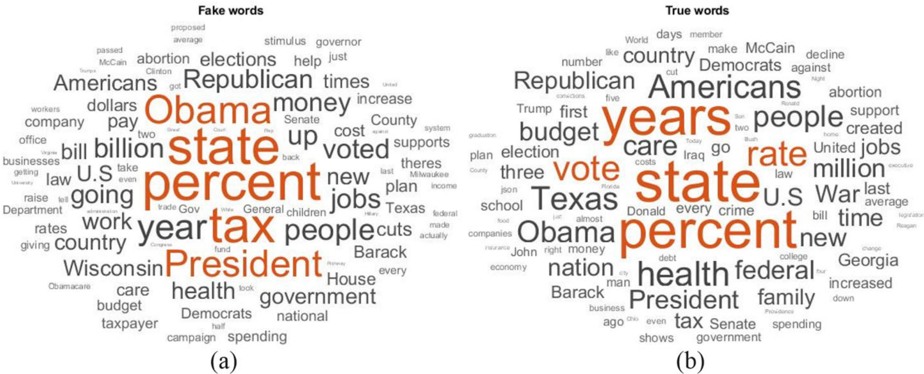


Fig. 3 LIDAR dataset Wordcloud of fake and true words

Table 3 Attributes in the dataset after feature extraction

ISOT		LIAR	
Attributes in the dataset	Attributes After Feature Extraction	Attributes in the dataset	Attributes after feature extraction
Title	Title	Id	Id
Text	Text	Title	Title
Subject	Subject	Statement	Statement
Date	Date	Subject	Subject
	Noun	Speaker	Speaker
	Verb	Speakers job title	Speakers job title
	Adjective	State info	State info
	Adverb	Party Affiliation	Party Affiliation
			Noun Count
			Verb Count
			Adjective Count
			Adverb Count

Prediction intervals for passive aggression follow the variance caused by increasing the maximum depth of the Naïve Bayes, and for deep neural networks, the result of several features evaluated are classified with fewer test set errors. The error rate of a dataset with missing data imputation was calculated with each classification technique proposed in this study, with results proportional to the test set error classified with MCAR perturbation as shown in Fig. 5(a). The error rate of a dataset without missing data imputation results in the non-compensative test set error with MCAR perturbation as illustrated in Fig. 5(b).

4.5 Evaluation metrics

The evaluation metric in this experiment is classification accuracy. Accuracy is the ratio of correct predictions to the total number of samples. Apart from accuracy, other performance

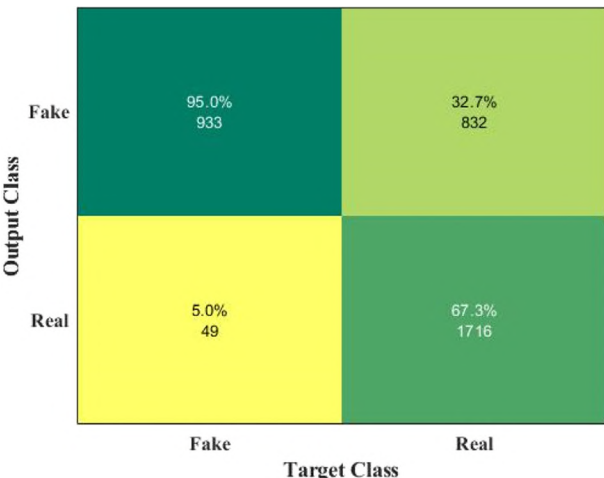


Fig. 4 Confusion matrix

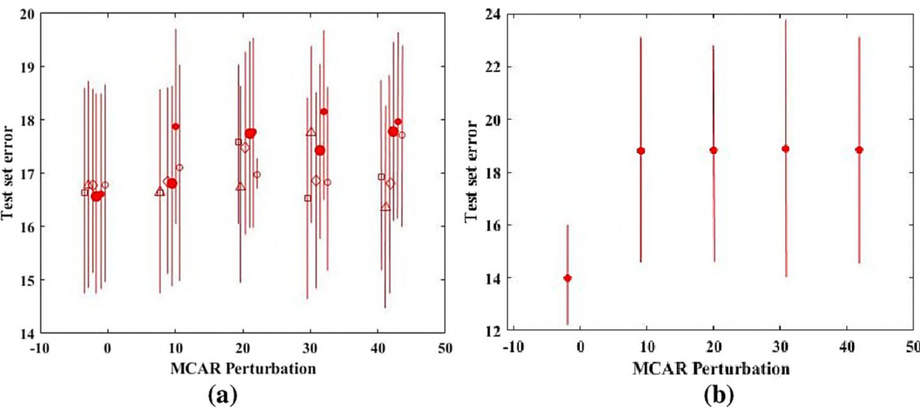


Fig. 5 Error rate of dataset (**a**) with and (**b**) without missing data imputation

measures, that is, True Positive Rate (TPR) also known as Recall, and F1 measures, are calculated based on equations below, respectively.

$$Accuracy = \frac{TP + TN}{TP + TN + FP + FN} \tag{26}$$

$$\text{Re}call = \frac{TP}{TP + FN} \tag{27}$$

$$F_1 = \frac{TP}{TP + \frac{1}{2}(FP + FN)} \tag{28}$$

Where TP represents the number of True Positive results, FP represents the number of False Positive results, TN represents the number of True Negative results, and FN represents the number of False Negative results.

The performance of the models was evaluated based on the predicted outcome values using common statistical measures. In this study, the evaluation metrics of Mean Absolute Percentage Error (MAPE), Residual Sum Of Squares (RSS) and AUC are computed for the forecasting models which is shown in Eq. (29).

$$MAPE = \frac{1}{N}\sum_{i=1}^{N}\left(\frac{\left(\left|\widehat{y_i} - y_i\right|\right)}{y_i}\right)*100\% \tag{29}$$

AUC is precisely the area under the ROC curve. An excellent system has an AUC close to 1 (it can perfectly distinguish between all fake and true news correctly), while a poor system has an AUC close to 0 (it would be considering all fake news as true and all true news as fake). AUC is more statistically consistent and more discriminating than accuracy, and it is usually applied in imbalanced classification problems, as is the case of fake news detection, where the number of ground truth fake news articles and true news articles have a very imbalanced distribution.

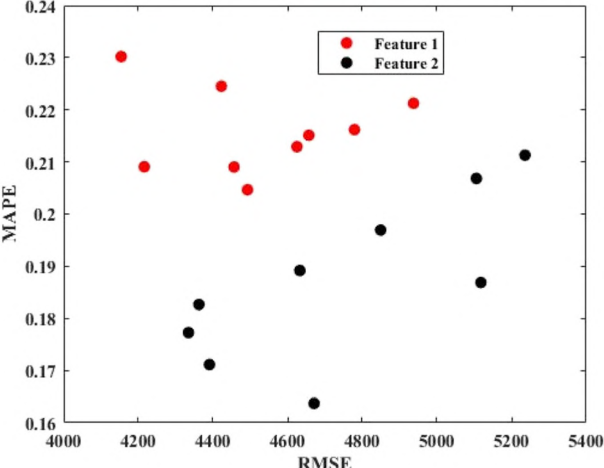


Fig. 6 MAPE performance analysis with RMSE

The MAPE performance result is demonstrated in Fig. 6. According to the result, as the number of features increases, the test error decreases. This indicates that the regression model gets more information from the additional features. However, after selecting the best features, adding more features decreases the model performance and increases the test error. This indicates that adding more features causes an overfitting problem due to the shared information within the features. Compared with selecting all 15 features, according to the result, selecting features reduces the test MAPE by about 5 cycles and test MAPE by about 0.23%. Therefore, in the following study, these selected features are used as the input features for the classification model.

Figure 7 depicts the performance analysis of the residual sum of square analysis. The residuals as an upright line connecting the actual values to the predicted value (red traces in the plot). The residual on that given datapoint is 0.5. However, if the scale meters, then that same datapoint has a residual of 7000. As calculated, the statistical analysis of the sum of all errors

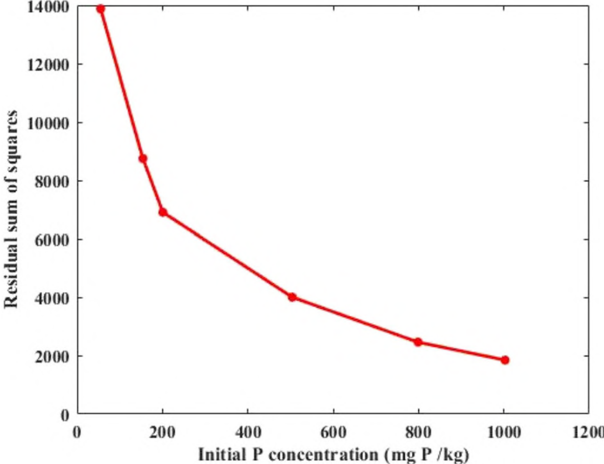


Fig. 7 Residual sum of square analysis

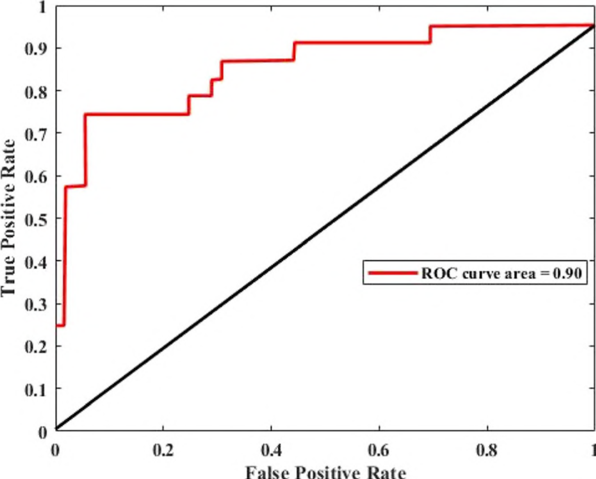


Fig. 8 ROC curve for fake news classifier

(the sum of residuals) is resulting in 0. This is because errors can positive or negative, as well the model underestimates and overestimates. By summing up the errors, all errors compensate for each other. This is a fundamental characteristic of the regression line and the method of least squares.

Figure 8 shows the Receiver Operational Characteristics (ROC) curve. The ROC curve focuses on the trade-off between the False Positive Rate (FPR) and the True Positive Rate (TPR) by adjusting the classification of each method. This proposed classifier got the highest accuracy score of 90%.

4.6 Outcome of classification

Table 4 represents the description of classification from the LIAR and ISOT dataset using the proposed classifier in view of the evaluation of accuracy, recall and F1 score. From this analysis, the dataset with barely true statements obtained a maximum of 26% as calculated by accuracy than other evaluation metrics, in before feature extraction. In after feature extraction, results in a maximum of 41% of recall as compared with others. In false statement analysis, the recall obtained a maximum of 49% in the evaluation before feature extraction and 50% in the evaluation after feature extraction. In a statement of half-true analysis, the accuracy results

Table 4 Classification report of LIAR dataset for proposed classifiers

Classifier	Label	Before feature extraction			After feature extraction		
		Accuracy (%)	Recall (%)	F1 Score (%)	Accuracy (%)	Recall (%)	F1 Score (%)
Multiple Classifier (Naïve Bayes, Passive Aggression,DNN)	Barely True	26	14	23	40	41	40
	False	24	49	30	43	50	47
	Half True	27	21	22	42	46	44
	Mostly True	27	28	30	39	51	43
	True	30	20	24	40	28	35

better with 27% of evaluation before feature extraction and in after feature extraction evaluation, recall obtains a maximum of 46%. In a mostly true statement, the F1 score obtains at 30% before feature extraction and 51% of the evaluation was obtained by recall in after feature extraction. Finally, the accuracy of 30% is achieved as a maximum before feature extraction for the true statement and 40% is the maximum accuracy obtained in after feature extraction. From this analysis, the analysis concludes that the calculation of multiple classifiers after feature extraction results better than before feature extraction.

The faster the training and prediction, the less time cost. The loss curves of the multiple classifications implemented in this study are shown in Fig. 9. It is obvious from the figure that the training loss and prediction loss were evaluated with similar training iterations of the data network. However, the loss of training and prediction of the classifiers have differed with fewer points of loss. Eventually, the prediction loss has been evaluated with minimum loss of curve as compared with the training loss.

To promote the work in this domain, more experiments were performed with the hybrid model trained on the ISOT dataset and tested on the LIAR dataset using the same configuration as before. ISOT is chosen for training because it is much larger and has minimum space for improvement since many models perform above the 0.9 classification accuracy threshold. Figure 10(a) and (b) show the ability of the ISOT-trained model to generalize on another dataset and plot the training and validation accuracy and loss values over the 10 epochs. Results show that while the training accuracy and loss are optimum after 6 epochs, the validation accuracy remains almost the same in all epochs and is lower than that achieved when training (and validating) on the LIAR dataset. The final performance after 10 epochs of training and testing on the whole LIAR dataset is shown in Fig. 10(b).

4.7 Comparison of proposed and existing methods

The proposed method of this study was compared with the existing methods such as CNN + LSTM [14] and Bi+LSTM+GRU [5]. Table 5 described the comparison of proposed and existing methods with various evaluation measures. From the table, the accuracy of the proposed and

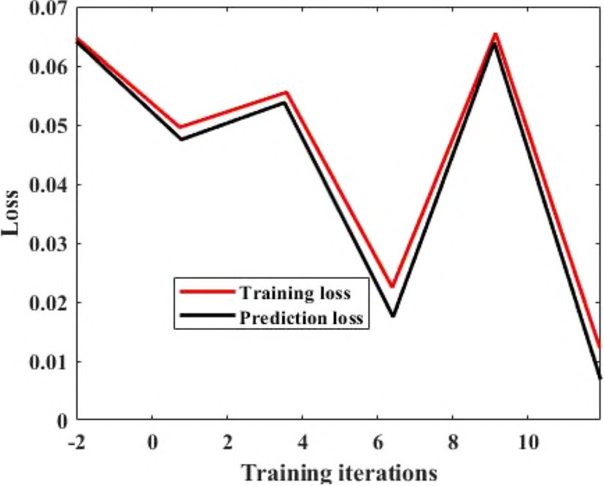


Fig. 9 Loss curve of multiple classifications

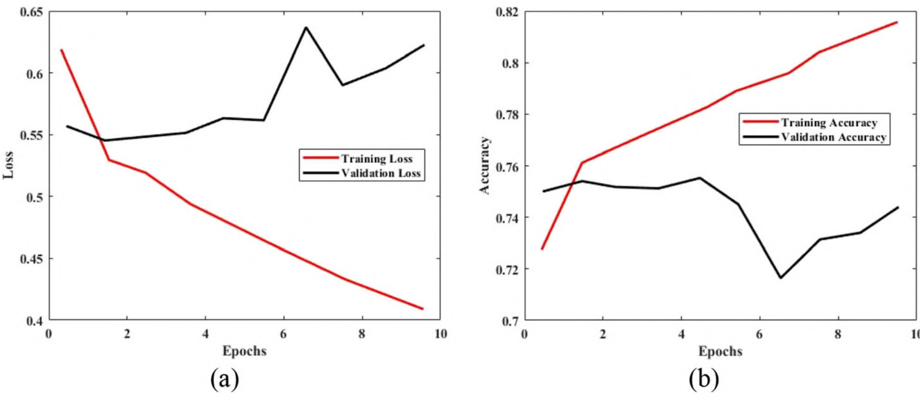


Fig. 10 Training and validation accuracy graph

existing methods is achieved to calculate maximum prediction analysis from the evaluation measures with percentage values of 99.8, 97.5, and 91.3. The recall and F1 score analysis are obtained better with the prediction of the proposed and the existing methods after the prediction analysis of accuracy. The average training time for the model without preprocessed input data was about 22 hours due to the more sequence length value. The preprocessed input data and the constraints helped reduce the sequence length to 120 from 200. The training time for the model was significantly reduced to 9 hours from 22 hours by a simple modification to the input samples and achieved the highest performance. Results showed the highest performance of 64 with 99.8% of accuracy and a corresponding F1-score of 95.6%. The results also indicate that input preprocessing helps increase performance with reduced training time. Accordingly, the time efficiency of the proposed framework is about 45 ms.

4.7.1 Ablation study of the proposed technique

To determine the relative importance of each module of the NPDNN, the article conducted a series of ablation studies on key parts of the model. The experimental comparison results are shown in Fig. 11.

This shows that these two attention mechanisms have a certain effect on our model performance. When the model used CNN-BiLSTM only, the performance of the model dropped by 2.6% to 3.2%, because the model loses very important propagation structure information. In addition, if only CNN-LSTM was used, the performance of the model dropped by about 8% on both data sets, because the model does not even read the text content of the news itself. However, the performance of Naïve Bayes on LIAR and ISOT data sets reached 8.83% and 8.92%, respectively, which proves that numerous clues can be used to detect fake news in the propagation structure.

Table 5 Comparison of proposed method and existing method

Evaluation measures	Proposed method	CNN+LSTM	Bi-LSTM-GRU
Accuracy	99.8%	99.7%	89.8%
Recall	98.9%	97.1%	91.6%
F1 Score	95.6%	93.8%	87.7%

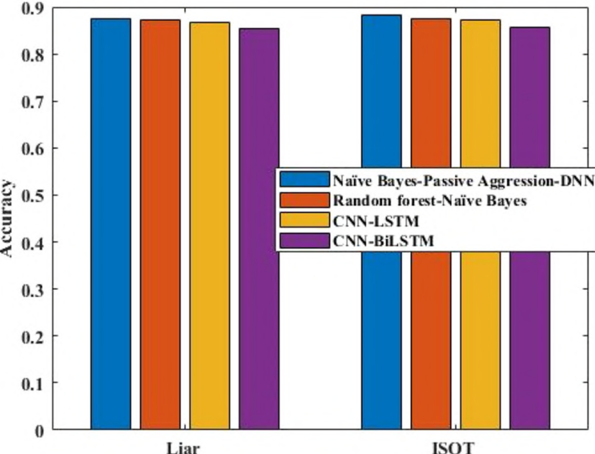


Fig. 11 NPDNN ablation analysis in accuracy

5 Conclusion

The fundamental purpose of this study is to mitigate one of social media's drawbacks: the rapid distribution of fake news, which frequently misinforms people, produces false impressions and harms society. In recent years, an increasing number of methods for automatically detecting false news have been presented in the literature. The datasets and a set of explicit classes are two critical aspects that have a substantial influence on the accuracy of the existing models. Therefore, this study proposed several models for the detection of fake news which have been highlighted as follows.

The study of fake news detection was carried out using the ISOT and LIAR datasets with real and fake news contents from Reuters.com, Politifact and FakeNewsNet.
Initially, the proposed technique selects important feature terms relying on the parts of speech (POS) in the textual information, and then uses sentiment analysis to estimate users' control variables for opinions using lexicon-based scoring analysis.
For improving classification-based false news identification, a data imputation preparation approach is presented. This approach is based on the utilisation of data imputation techniques to handle missing values in a dataset.
Subsequently, the term frequency and inverse document frequency (TF-IDF) were used for the extraction of useful features from the datasets to help the detection accuracy.
Finally, the fake news was detected using multiple classification models. Initially, for the multiclass prediction and robustness of predicting the class of text, the Naïve Bayes model had been used. Secondly, the passive-aggressive classifier trains the model incrementally and eventually, the deep neural network was used to increase the efficiency to detect fake news.

The outcomes of this study revealed that the suggested method's overall evaluation achieved a 99.8% accuracy rate for detecting false news. The findings are compared with and without multiple imputation execution in the creation of multiple classifier calculations for test set errors. Using the developed technique, this study produced a higher prediction rate while

evaluating various statements from the dataset, such as barely true, half true, true, largely true, and untrue. Finally, the developed strategy's performance is compared to that of current methods, in which the proposed method proved to be more efficient. The proposed classification models paired with the suggested missing data variable models and feature extraction strategy outperforms baselines, according to experimental results. Certainly in future work, it would be fascinating to test this proposed strategy for false news identification on other data sets.

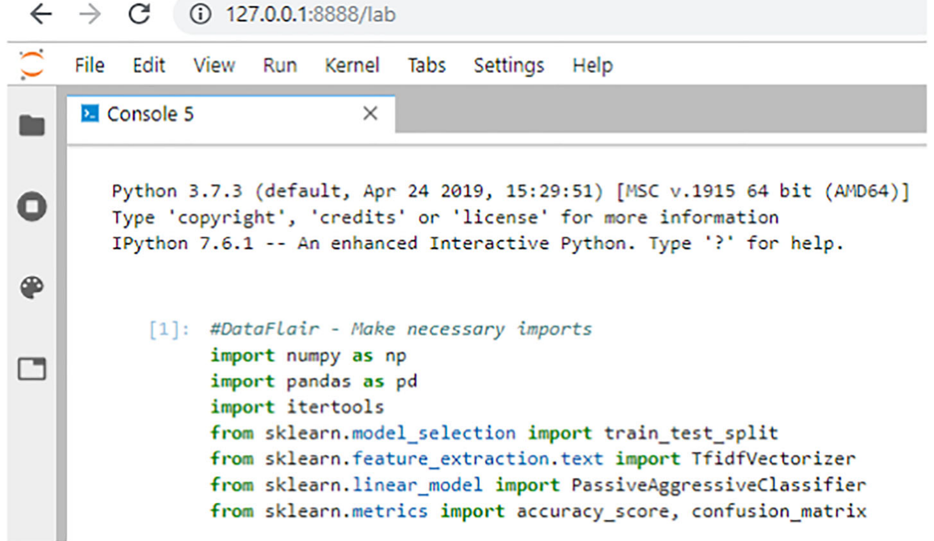
Appendix

```
clc;
clear;
close all;
warning off;
%% read data
global compoundScores sFeat yvalid xvalid
T=readtable('2020-04-21 Coronavirus Tweets.csv','TextType','string');
T=T(1:1000,1:22);
dat=(T(:,end));
Var=dat.Properties.VariableNames;
emb=readWordEmbedding('glove.6B.100d.txt');
[compoundScores,documents]=preprocessing(dat,Var,emb,T);
%% n gram model
bag = bagOfNgrams(documents);
vec2=tfidf(bag);
full_dat =full(vec2(:,:));
%% feature selection using hybrid TSA-HHO-SA
SearchAgents=5;
Max_iterations=10;
lowerbound   = 0;
upperbound   = 1;
dimension = size(full_dat,2);
fitness=@jFitnessFunction;
ho = 0.3;
% Hold-out method
HO = cvpartition(compoundScores,'HoldOut',ho);
[Best_score,Sf,concurve]=tsa_hho_sa(SearchAgents,Max_iterations,lowerbound,upperbound
,dimension,fitness,full_dat,compoundScores,HO);
figure;
plot(concurve,'-b','linewidth',2);
xlabel('Iterations');ylabel('Objective value');
sFeat = full_dat(:,Sf);
sFeat=sFeat(:,1:50);
xtrain = sFeat(HO.training == 1,:);
ytrain = compoundScores(HO.training == 1);
xvalid = sFeat(HO.test == 1,:);
yvalid = compoundScores(HO.test == 1);
```

```
%% hybrid BOA artificial neural network
N_iter=50;
Lb=-1;
Ub=1;
n=5;
fobj=@fitnANN;
accuracy=ANN_BOA(xtrain,ytrain,N_iter,Lb,Ub,n,fobj);
function [X]=initialization(N,dim,up,down)

if size(up,1)==1
   X=rand(N,dim).*(up-down)+down;
end
if size(up,1)>1
   for i=1:dim
      high=up(i);low=down(i);
      X(:,i)=rand(1,N).*(high-low)+low;
   end
end
end
global net yvalid xvalid compoundScores sFeat
net = feedforwardnet(10);
net.trainParam.epochs=100;
net = train(net, sFeat', compoundScores');
mdl=fitctree(xvalid,yvalid);
y = net(xtrain');
acc=length(find(round(y')==ytrain))/length(ytrain);
weights = getwb(net);
dim=size(weights,1);
[fmin,best_pos,Convergence_curve]=BOA(n,N_iter,Lb,Ub,dim,fobj);
net = setwb(net,best_pos);
y = net(xvalid');
y=round(y);
y=predict(mdl,xvalid);
accuracy=length(find(yvalid'==y))/length(y);
d1=find(y==0);
d2=find(y==1);
d3=find(y==2);
```

Snapshot of the Run Image



Data availability Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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secure authentication protocol for healthcare service in IoT with Q-
et based secret key generation

Cite

Article type: Research Article

Authors: Mahajan, Rupali[a; *] | Chavan, Smita[b] | Ajalkar, Deepika Amol[c] | SV, Balshetwar[d] | Khadkikar, Prajakta Ajay[e]

Affiliations: [a] Department of Computer Engineering, Vishwakarma Institute of Information Technology, Kondhwa (BK), Pune 411048, India | [b] Department of Information Technology, Government College of Engineering Aurangabad, India | [c] Department of Information Technology, G. H. Raisoni College of Engineering and Management, Pune, India | [d] Department of Computer Science and Engineering, Faculty of Engineering, YSPMs Yashoda Technical Campus, India | [e] Department of Computer Engineering, SCTR's Pune Institute of Computer Technology, Pune, India

Correspondence: [*] Corresponding author. E-mail: rupali.mahajan@viit.ac.in.

Abstract: The major intention of this research is to propose a secure authentication protocol for healthcare services in IoT based on a developed Q-Net-based secret key. Nine phases are included in the model. The sensor node, IoT device center, gateway node, and medical professional are the four entities involved in the key generation process. The designed model derived a mathematical model, which utilized hashing function, XOR, Chebyshev polynomial, passwords, encryption algorithm, secret keys, and other security operations for performing effective authentication. Here, the secret key is generated with the Deep Q-Net-based sub-key generation approach. The proposed method achieved the minimum computation time of 169xe9 ns, minimum memory usage is 71.38, and the obtained maximum detection rate is 0.957 for 64 key lengths. The secure authentication using the proposed method is accurate and improves the effectiveness of the system's security.

Keywords: Authentication, Healthcare Service, Internet of Things, data security, secret key generation

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A Review on AI based Restaurant Management System

Mr. Abhishek Korde[1], Ms. Aditi Loni[2], Ms. Vaibhavi Deshpande[3], Ms. Dhanashri Rajput[4], Ms. Rutuja Chavan[5], Mrs. Dr. S. V. Balshetwar[6].

[1,2,3,4,5]*UG Student, Computer Science & Engineering,*
Yashoda Technical Campus, Satara, Maharashtra, (India)
[6]*Associate Professor, Head of Department, Computer Science & Engineering,*
Yashoda Technical Campus, Satara, Maharashtra, (India.)

Abstract

Food industry being 7th largest industry, we are developing an website where a user can view number of Cafe's & Restaurants, Monitor bookings, pre-decor for celebrations, order food, get mapped to that location and other various activities all under one platform-"Online Table Reservation System". It is a web application which consists of four interfaces viz. Admin, User, Order & Table booking, and Payment gateway. User first needs to sign up and then log in to access booking and ordering. They have also added in gallery to excite our users. Technologies used in project are Machine Learning for Recommendation System, Web Development, Android Development, Etc.

Keywords: *AI chatbot, Food Industry, Machine Learning, Online Reservation, Web Application.*

1. Introduction:

There has been vast growth in the Food Industry with help of leading technologies. The Hotel industry is one of the growing sectors of world. This system is generally made for Time saving purpose. Pre Booking Table and ordering food is main objective of this system. When it comes to food and dining, people always look for quality and variety of food and restaurants. In traditional booking customer used to make a phone call and paper work to book table. But with this system, user can make his order within minutes on his finger tips. This system not only saves time but also gives chance to explore several café/restaurants in town with online as well offline payment methods. Customer feedback is must so; this system also has feedback section where people can give there review on food and experience.

1.1. Literature Survey:

1	Restaurant table	2018	Shaziaiaz, reservation system using Amnanisar.	The Key of paper was to allow the management administration and employees of restaurant to grip the customers to place their orders and to find free tables. According to their required

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				android mobile No. of seats. RTRSMA app will enable the user to access and manage the arrangement of the tables and foods. The general objective of RTRSMA was to build up reservation system for table reservations to assist workers with solving basic issue with menu reservation system.
2	Design and implement an online restaurant reservation system	2018	Acheampong Samuel	As stated earlier, customers basically order food or reserve tables by means of their PCs, mobile devices as well as other portable devices such as Tablets. Customers order food using computers via browsers like Mozilla Firefox or via custom apps. This basically is the main trend with regards to food Ordering and table reservation in the restaurant industry. In spite of consumer demand, restaurants' use of technology remains in its infancy hence, the use of technology in the restaurant industry is expected to gain dominance and recognition in the future.
3	Digital table booking and food ordering system using android application	2014	Surabhi Thakar, Prajakta Kulkarni, Rasika Thorat.	In This paper, some form of static menu is utilized to convey the available food and beverage choices to customers. Said menus are generally photo based and hence impose restrictions on the textual real estate available and the ability is saturator has to update them. This application specifies the requirements for a restaurant digital menu and ordering replacement strategy to alleviate the problems associated with the current archaic method. Three related concepts are encompassed by the general scope of the Restaurant Menu and Ordering System. The first pertains to their placement of photo menus using an electronic format, the second relates and the third surrounds the process of transferring said

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				electronic orders to the kitchen for preparation.
4	Restaurant Table Reservation System Using Android Mobile Application(RTRSMA)	2018	Hafiza Mahrukh Shahzadi, Ms.Shazia Riaz, Ms.Amna Nisar	The project has concluded that if a customer is willing to visit the restaurant and he finds no table is available for the dinner/lunch then he/she has to wait long for the table availability. With the help of this app user can choice the table's location according to their need and willing e.g. Table can be reserved as according to number of visitors. Moreover, you can easily book the hall for a celebration party or any mega event and can see pictures of interior from the App. Keeping in view the demand of proposed project that gives a series of services and provides the customer to easily book hall or to reserves their available table without waiting through an android app. In this particular project we have resolved issues being faced by Quilim restaurant located in Faisalabad by developing app named as, Quilim APP" that can be downloaded and then just update his/her selfdata and can have access to latest news and menu with the restaurant. This app will get its importance as now days more and more people are getting into android and fast life.
5	Digital Food Ordering System using Android for Mess	2019	Pandhare Sonalil, Shrike Priyanka, Swami Megha, Takawane Pratima.	This application developing an online mess service booking system based on user's location where users are able to sort messes according to their requirement. This system is to increase efficiency and reduce human errors and provide high quality services. By using this system, this will avoid long queues at the counter due to the speed of execution. This system is time-saving. The customer meal service android application can handle the billing hence it is the modern way to grow up the business using E-commerce. A system is able to stand out from

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				competitors in the food service industry. Here the user is permitted to rate their favorite mess and give feedback to improve and encourage their experiences with mess providers. Therefore, the conclusion of the proposed system is based on customer's requirement. The wide range of people can use this system if they know how to operate the android Smartphone. The scope of the proposed system is justifiable because in large amount peoples are shifting to many cities so a wide range of people can use this system.
6	Automated Restaurant Management System	2016	Aman Jain, Snehal Chauhan, Anish Hirlekar, Suraj Sarange.	Automated Restaurant Management System will work as a link between man machine to provide optimum quick and effective and almost effortless services to the hotel and hospitality industry. It is a low power system which will not only reduce man power required but also reduce the possibility of human errors. It is cost effective as it involves one time investment. The maintenance cost will be considerably low as compared to the salary of the waiters. Automated Restaurant Management System will revolutionize the hotel industry.
7	Design and Implementation of Digital Dining in Restaurants using Android	2014	Shobhit Goyal1 , Meenu Bhati	In this paper, we present an automated food ordering system with-real time customer feedback (AOSRTF).This system is convenient, effective and easy thereby improving the performance of restaurant’s staff. It will also provide quality of service and customer satisfaction. Overall conclusion is that, this is a fabulous food ordering system for the restaurant sector, made by combining the Android and Wireless technology.

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8	A Review Paper on Online Restaurant Management System	2017	Prof. N. M. Yawale, Prof. N. V. Pardakhe, Prof. M. A. Deshmukh, Prof. N.A. Deshmukh	Here the need for tablet food ordering is analyzed and its advantages over the traditional food ordering system in restaurants are studied. The proposed online restaurant management system is time saving and error free as compared to the traditional system. This system attracts customers and also adds the efficiency of maintaining the restaurant's ordering and billing. Hence it is the modern way to grow up the business using E-commerce. Here implementation of an advanced e-restaurant menu ordering system using smart android mobile Phone. This system entirely reduces the unnecessary time. Every order is associated with an individual seat at the table, and orders are built one customer at a time, just like on paper, but with greater accuracy. Items can also easily be shared by the whole table, moved or modified, and noted and the cost can be calculated in real time. The idea of the advanced e-restaurant can also be extended for future using GPRS module. GPRS module can be used to monitor and request of the menu order from table will be directly sent to the predefined web link for process of even billing the items purchased.
9	Table booking and restaurant management system using android application (OPSS)	2020	B. Naveen Kumar, B. Sai Varun	To conclude that this restaurant management android application is implemented to reduce the manual process for both customer and management of the restaurant and to make the work look professionally. The manual system which were used in the past are do not serve the customer in the best possible way and the data can be edited but the new proposed application will have correct records of data and have no authority to edit or manipulate the data.

1.3. Literature Survey:

As stated in (1), Customers do have lot of expectations while handling and booking things online. This app will provide vast variety of facility from parking slots o payment, the main aim of this app is to provide customer ease and less timeconsuming reservation. By storing the contact details of customers, they will give alerts regarding discounts, offers and occasions, etc. Visitor will be able to make their own choice about food, table, and place and also inform about guests expected.

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(2) The potential of technological advantages generated out of the implementation of artificial intelligence (AI) and robotics in hotel operation are considered closely by the industry players (Reis et al., 2020). The impactful implementation of AI and robotics in hotel operations has been studied by several researchers (Tussyadiah, 2020). The implementation of these two technologies can help in the protection of the guests and the service personnel. Some of the early adopters of these technologies are situated in some of the technologically advanced countries like Japan, (Reis et al., 2020). Japan has produced the fully automatic hotel run by robots named Henn-na, (Fusté-Forné& Jamal, 2021; Tung & Law, 2017). The other technologically advanced countries like Taiwan have been intensifying their hotel operations with the help of robotics.

(3) In this proposed system, user has to order or book table through the app, and has to pay 50 percent of order in advance as a confirmation. This app helps people to explore various restaurants and food dishes on finger tips. Customer can also cancel the booking and get refund if failed to arrive. The project is based on Android application.

(4) To conclude that this restaurant management android application is implemented to reduce the manual process for both customer and management of the restaurant and to make the work look professionally. The manual system which were used in the past are do not serve the customer in the best possible way and the data can be edited but the new proposed application will have correct records of data and have no authority to edit or manipulate the data.

(5) As discussed, this providesfacility customers to reserve tables for dining, and can also get details of hall availability for reservation of party and celebration. Also this will allow the hotel manager to manage the services, feedback and make changes in menu. The main motto of this system is to provide hall for celebrations and parties. This application will have two main module application one for user customers and other for hotel admin.

(6) As the information technology is taking lead, the internet usage is vastly increased and people have become ‘mobiholic’. Earlier, people used to visit the restaurant and then order table, but now it’s made online. The primary aim of the mobile based customer care service is for reservation, table management and customer management software for restaurants though web. This web application is based on Apache Web Server and used MySQL for backend. PHP as Web Programming language.

(7) The purpose of this project was to manage the crowd and eliminate the problem of waiting time. This system helps customer to book food from home itself without have to wait for long hours and system itself tells with help of time – series algo informs the available table at time. Also, this system notifies customer if any delay. And also manage large number of public.

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(8) The technologies used here are web development by using Java, MySQL. They have also made use of JSP technology in system. The objective of this project was to build a system that can overcome problem of Low-quality delivery and old manual / paper reservation systems.

(9) Online reservation system companies configure the information technology aspect of managing reservations and a way to market your restaurant out to the public. They are not just a place to book a reservation, but a search database for potential guests to find the right restaurant for the occasion, in right location, and right time. Every person performs some information search prior to a purchase. Consumers acquire information as a way of reducing the risk in the event of uncertainty regarding the outcome of an action. A complete information search will greatly lessen the consumer's difference between external and internal information (Locander& Herrmann 1979). By reducing the risk, the consumer will generally increase their satisfaction, which is the primary goal of every consumer.

(10) Discussion of restaurant management mainly focuses on price management and meal duration management (Kimes, 1999). For price management, restaurants can use price setting or promotion discounts to shift excess demand from peak periods to elsewhere and thus serve more customers overall. InSusskind, Reynolds, and Tsuchiya's survey(2004), p. 284 out of 367 respondents indicate willingness to transfer to off-peak periods for dinner if incentives are provided. Such moves can help relieve long waiting at peak time and bring extra revenue at off-peak. These studies indicate that directing customer segments to different dining periods to generate higher revenue is feasible.

1.4. Conclusion:

The purpose of the wireless restaurant management system is to improve worker efficiency and to maximize profit margin of restaurant owners by providing better service. Providing prompt response to customers through use of a System and data collection by the Main Dispatcher will allow this to happen. This project proved to be a larger task than expected due to lack of manpower and late arriving parts. Certain functionality also had to be abandoned to meet time constraints. The System is not designed to replace the existing ordering systems which are at many restaurants but to complement it. Once the Restaurant Management System becomes further refined with the ideas discussed in the previous section, it will pose to be an indispensable tool.

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REVIEW ON SORTING TECHNIQUES VISUALIZER

Dr.S.V.Balshetwar[1], MuskanHanif Shaikh[2], AartiMadhukar Palande[3], Aishwarya Anand Kumbhar[4], Rutuja Arjun Mane[5]

[1,2,3,4,5]*Computer Science and Engineering, Yashoda Technical Campus ,Satara, (India)*

ABSTRACT

The purpose behind this project is to study how to perform different operations of sorting algorithm of data structure so student can easily learn various types of algorithm through an graphical view it will make a data structure learning more interesting. Data Structure design and analysis of the algorithm is big challenge for both computer and Science Students. Implementation of this project to make clear understanding of various algorithm of data structure such as an Bubble sort, Insertion sort, Selection Sort and so on .The various tools is used for the study are case analysis of sorting algorithm such as best case average case.

Keywords *- Analysis of Sorting Algorithm, Selection Sorting Algorithm Visualization, Sorting Visualizer, Visualization of Sorting Technique, Visualizing Sorting Algorithm.*

I. INTRODUCTION

Data structure and algorithms (DSA) is important field of Computer Science and Engineering. Data structure related concepts are complicated to understand for learners so this project performs visualization of algorithms. It helpful for students to understand that how actually sorting methods work. Methods are like Bubble sort, Selection sort, Insertion sort, Merge sort and so on. In visualization data can be represented by Bar graph. Animation tool shows sorted data and unsorted data with different colors. Colors change after sorting techniques. This platform helps to improve theoretical concept regarding Data structure and algorithm.

LITERATURE SURVEY

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1.	A system for algorithms' animation	1999	D. Merlini, S. Petruzzi, R. Sprugnoli and M. C. Verri	Data structure related complicated concepts are difficult to understand for student. So, visualization technique helps teacher to teach them very easily Sorting algorithm show user input data through animation. This tool shows sorted data and unsorted data with different color. It helps student to understand, how data can be sorted dynamically.
2.	AVE: A Dynamic Algorithm Visualization Environment for Novice Learners	2008	E. Vrachnos and A. Jimoyiannis	Sorting algorithm represent sorted array through animation. This tool shows sorted data and unsorted data with different color. It helps student to see how sorting function actually work to sort data at backend
3.	Sorting Algorithm visualizer	2022	Thakkar, Kavita, S. Dashand S. K. Joshi	It is E-learning platform which helps to improve theoretical concept regarding Data structureand algorithm. Data can be represented by Bar graph, and then sorting algorithmmay be apply on that.In Sorting Visualizer, take input data from user and show that data as bar graph. Then choose animation tool and after that algorithm can be apply on it.
4.	Algorithm Visualizer: features and Its working	2021	Goswami, A. Dhar, A. Gupta and A. Gupta	Some learners can't understand theory with clarity. From this work student can visualize several algorithms and learn new concepts. This visualizer is easy to operate and implement. It contains stepwise representation of visualization of algorithm which makes it easy to understand.

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5.	Creating Engaging Online Learning Material with the JSAVJavaScript Algorithm Visualization Library	2016	V. Karavirta C.A.Shaffer and	DSA is complicated to learn for most of the students. Learners can improve their DSA topics with JavaScript Algorithm Visualization. In this contain JavaScript Algorithm Visualization library. This library helps to visualize data structures algorithms. Learners can see previous step of visualization with actual current step.
6.	Visualizing Sequence Of Algorithms For Searching and Sorting	2009	Bremananth R.,Radhika V.ThenmozhiS.	Visualizing sequence of algorithm for searching and sorting in this paper. It help to understand how perform the sorting method in easy way. The main pros of algorithm visualization is acquire the knowledge through performing with set of data, technique of manage time and use of memory. It shows nine type of sorting algorithm form this one of animation system is BALSA(Brown Algorithm andanimator).
7.	Interactive visualization of high dimensional marketing data	2015	Alfa Yohannis Yulius Prabowo	Interactive visualization, it is used to associate all types of variable and also describe visualization of huge amount of data for financial organization. We use matrix visualization as a selection tool, its simple for find the data. The clients select data of selected variable and start analysis by using 5 variable. Using this we give good customer behavior knowledge.

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8.	Sorting visualization using ViSA (visualization of sorting algorithm)	2012	Tihomir Orehovački	In this paper, ViSA (Visualization of sorting algorithm) it describe a tool and different type of technique for visualization of algorithm. The ViSA is simple and self operating visualization system, it explain and compare step by step every sorting algorithm which is more useful to every educational student for analyze the sorting algorithm also for faculty member for teaching.
9.	Sort Attack: Visualization and Gamification of Sorting Algorithm Learning	2015	Alfa Yohannis, Yulius Prabowo	Sorting algorithm is perform fundamental role in the field of computer science, which are not understandable to everyone to defeat this problem it has been moving toward the game visualization. The combining algorithm gaming tool and instructional or graphical design isan minor task, so it require attentive design, because of that it developed conceptual model for learning instruction and Improve visualization learning.
10.	Comparative of Advanced Sorting Algorithms Based on Time and Memory Usage	2021	MarcellinoMarcellino, Davin William Pratama Kristien Margi	In this paper, it compare different type of advance sorting algorithm such as Quick sort, Heap sort, Merge sort, insertion sort. A algorithm compare on the bases of time and memory require to perform data sorting. Applications write using VS code and satisfy using python language. In this it show introspective sort good at

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				time and heap sort is good for memory.
11.	Analysis of Algorithm Visualizer to Enhance Academic Learning	2022	G. Prabhakar, S. Gaur, L. Deshwal and P.Jain	This displays how algorithms work in graphical way. Analysis of algorithm used to improve qualityof education. It is better way for students to grasp the knowledge of data structure and algorithms. In this contain download visualizerbutton. After clicking this button we can perform it on offline in our personal computers (PCs).
12.	Visualize and Learn Sorting Algorithms in Data Structure Subjecting a Game- based Learning	2022	W. H. Lim, Y. Cai, D. Yao and Q. Cao	Game based learning is important role in sorting visualization. Teacher teach them student through experiment of game playing, to clear the concept of students very easily. Student can grasp the working of data structure and time complexity ofthat algorithm in experimental way.
13.	Algorithm Visualization Environments: Degree of interactivity as an influence on student-learning	2020	P.RozaliaOsztián, Z. Kátaiand E. Osztián	Sorting visualization is e-learning platform, to improve learning environment through visualization of sorting algorithm. It improve the additional feature of online learning such as notes, data structure related material etcthrough visualization

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14.	AlCoLab: Architecture Of Algorithm Visualization System	2008	C. Foutsitzisand S. Demetriadis	This system is related to education system. The purpose of this system is to enhance learning. This visualization increases better understanding of algorithms.
15.	A New Network Topology Visualization Algorithm	2011	Y. Guozheng, L. Yuliang and C. Huixian	In this paper contain network topology Visualization. It shows different network topology visualization algorithm. There are two policies. First layer contain network characters and second layer contain dynamic layout process.
16.	Experimental study on the five sort algorithms	2011	You Yang, Ping Yu and Yan Gan	In this there are five sorting algorithms used such bubble sort, select sort, insertion sort, merge and quick sort. Along with this time complexities summarized here. Five sort algorithms were selected to do the experiments. Because of that there were manyvariations of these algorithms, therefore the algorithms definedfirstly in the beginning section, then the performances of the algorithms given by experiments in the next section.
17.	A new modified sorting algorithm: A comparison with state of the art	2017	F. Idrizi, A. Rustemi and F.Dalipi	Here analysis and comparison between the state of sorting algorithm done, based on their analogical functionalities, as well here they give descriptions of modified algorithm and finally give conclusions about the performance.

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18.	Super Sort Sorting Algorithm	2018	Y. Gugale	The super sort sorting algorithm proposed in this paper is based onthe principle of selecting the sequence of already sorted elements in a given unsorted list. It is sequence of sorted elements in an array of random numbers soas to reduce the number of steps needed to sort.
19.	Design Patterns for Sorting Algorithms	2019	J. A. Rosiene and C. P. Rosiene	Here teaching of algorithms done by design patterns rather than only implementation here student can explore new realization.
20.	Mid–Point Sorting Algorithm: A NewWay to Sort	2022	A. Garg, V. Patel and D. Mishra	This paper presents a new sorting algorithm that takes into account the mid-point of the unsorted array and checks for its neighbors in every iteration

II. CONCLUSION

It is teaching support application which visualizes the most known sorting algorithms. User run sorting algorithm by giving custom array. We tried to create high-quality software with a user-friendly and easy-to-use interface, which could be used by lecturers, tutors, and students. Possible next improvement of the applications is extension it by other algorithms.

III. ACKNOWLEDGEMENT

IV. We take this opportunity to express our deep sense of gratitude to our guide, Dr.S.V.Balshetwar Head of Computer Engineering Department, for her continuous guidance and encouragement during this study. Without her valuable suggestion and encouragement this would not have been possible.

We find our self spellbound to acknowledge thanks to our Father and Mother for their silent support, patience, encouragement and affection without which this work would never have been possible

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[1,2,3,4,5]*Computer Science and Engineering, Yashoda Technical Campus ,Satara, (India)*

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4.	Algorithm Visualizer: features and Its working	2021	Goswami, A. Dhar, A. Gupta and A. Gupta	Some learners can't understand theory with clarity. From this work student can visualize several algorithms and learn new concepts. This visualizer is easy to operate and implement. It contains stepwise representation of visualization of algorithm which makes it easy to understand.

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8.	Sorting visualization using ViSA (visualization of sorting algorithm)	2012	Tihomir Orehovački	In this paper, ViSA (Visualization of sorting algorithm) it describe a tool and different type of technique for visualization of algorithm. The ViSA is simple and self operating visualization system, it explain and compare step by step every sorting algorithm which is more useful to every educational student for analyze the sorting algorithm also for faculty member for teaching.
9.	Sort Attack: Visualization and Gamification of Sorting Algorithm Learning	2015	Alfa Yohannis, Yulius Prabowo	Sorting algorithm is perform fundamental role in the field of computer science, which are not understandable to everyone to defeat this problem it has been moving toward the game visualization. The combining algorithm gaming tool and instructional or graphical design isan minor task, so it require attentive design, because of that it developed conceptual model for learning instruction and Improve visualization learning.
10.	Comparative of Advanced Sorting Algorithms Based on Time and Memory Usage	2021	MarcellinoMarcellino, Davin William Pratama Kristien Margi	In this paper, it compare different type of advance sorting algorithm such as Quick sort, Heap sort, Merge sort, insertion sort. A algorithm compare on the bases of time and memory require to perform data sorting. Applications write using VS code and satisfy using python language. In this it show introspective sort good at

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				time and heap sort is good for memory.
11.	Analysis of Algorithm Visualizer to Enhance Academic Learning	2022	G. Prabhakar, S. Gaur, L. Deshwal and P.Jain	This displays how algorithms work in graphical way. Analysis of algorithm used to improve qualityof education. It is better way for students to grasp the knowledge of data structure and algorithms. In this contain download visualizerbutton. After clicking this button we can perform it on offline in our personal computers (PCs).
12.	Visualize and Learn Sorting Algorithms in Data Structure Subjecting a Game- based Learning	2022	W. H. Lim, Y. Cai, D. Yao and Q. Cao	Game based learning is important role in sorting visualization. Teacher teach them student through experiment of game playing, to clear the concept of students very easily. Student can grasp the working of data structure and time complexity ofthat algorithm in experimental way.
13.	Algorithm Visualization Environments: Degree of interactivity as an influence on student-learning	2020	P.RozaliaOsztián, Z. Kátaiand E. Osztián	Sorting visualization is e-learning platform, to improve learning environment through visualization of sorting algorithm. It improve the additional feature of online learning such as notes, data structure related material etcthrough visualization

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14.	AlCoLab: Architecture Of Algorithm Visualization System	2008	C. Foutsitzisand S. Demetriadis	This system is related to education system. The purpose of this system is to enhance learning. This visualization increases better understanding of algorithms.
15.	A New Network Topology Visualization Algorithm	2011	Y. Guozheng, L. Yuliang and C. Huixian	In this paper contain network topology Visualization. It shows different network topology visualization algorithm. There are two policies. First layer contain network characters and second layer contain dynamic layout process.
16.	Experimental study on the five sort algorithms	2011	You Yang, Ping Yu and Yan Gan	In this there are five sorting algorithms used such bubble sort, select sort, insertion sort, merge and quick sort. Along with this time complexities summarized here. Five sort algorithms were selected to do the experiments. Because of that there were manyvariations of these algorithms, therefore the algorithms definedfirstly in the beginning section, then the performances of the algorithms given by experiments in the next section.
17.	A new modified sorting algorithm: A comparison with state of the art	2017	F. Idrizi, A. Rustemi and F.Dalipi	Here analysis and comparison between the state of sorting algorithm done, based on their analogical functionalities, as well here they give descriptions of modified algorithm and finally give conclusions about the performance.

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18.	Super Sort Sorting Algorithm	2018	Y. Gugale	The super sort sorting algorithm proposed in this paper is based onthe principle of selecting the sequence of already sorted elements in a given unsorted list. It is sequence of sorted elements in an array of random numbers soas to reduce the number of steps needed to sort.
19.	Design Patterns for Sorting Algorithms	2019	J. A. Rosiene and C. P. Rosiene	Here teaching of algorithms done by design patterns rather than only implementation here student can explore new realization.
20.	Mid–Point Sorting Algorithm: A NewWay to Sort	2022	A. Garg, V. Patel and D. Mishra	This paper presents a new sorting algorithm that takes into account the mid-point of the unsorted array and checks for its neighbors in every iteration

II. CONCLUSION

It is teaching support application which visualizes the most known sorting algorithms. User run sorting algorithm by giving custom array. We tried to create high-quality software with a user-friendly and easy-to-use interface, which could be used by lecturers, tutors, and students. Possible next improvement of the applications is extension it by other algorithms.

III. ACKNOWLEDGEMENT

IV. We take this opportunity to express our deep sense of gratitude to our guide, Dr.S.V.Balshetwar Head of Computer Engineering Department, for her continuous guidance and encouragement during this study. Without her valuable suggestion and encouragement this would not have been possible.

We find our self spellbound to acknowledge thanks to our Father and Mother for their silent support, patience, encouragement and affection without which this work would never have been possible

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Description

Notification System Using Cloud

Prof. Jagtap K.P, Taralekar Shubham Mahesh, Ithape Prasanna Manikrao, Pawar Shubham Chandrakant, Bagwan Akib Altaf

Professor, Student, Department of Computer Science and Engineering,

Yashoda Technical Campus Wadhe, Satara.

ABSTRACT:

The notification system is a project created by our group. The main objective of the Notification System project is to provide a system that manages the records of students and sends notifications or any notifications related to the campus This project maintains the records of students and campus companies. This project keeps records details etc. It represents the records of each department.

KEY WORD: Notification system, web services. API.

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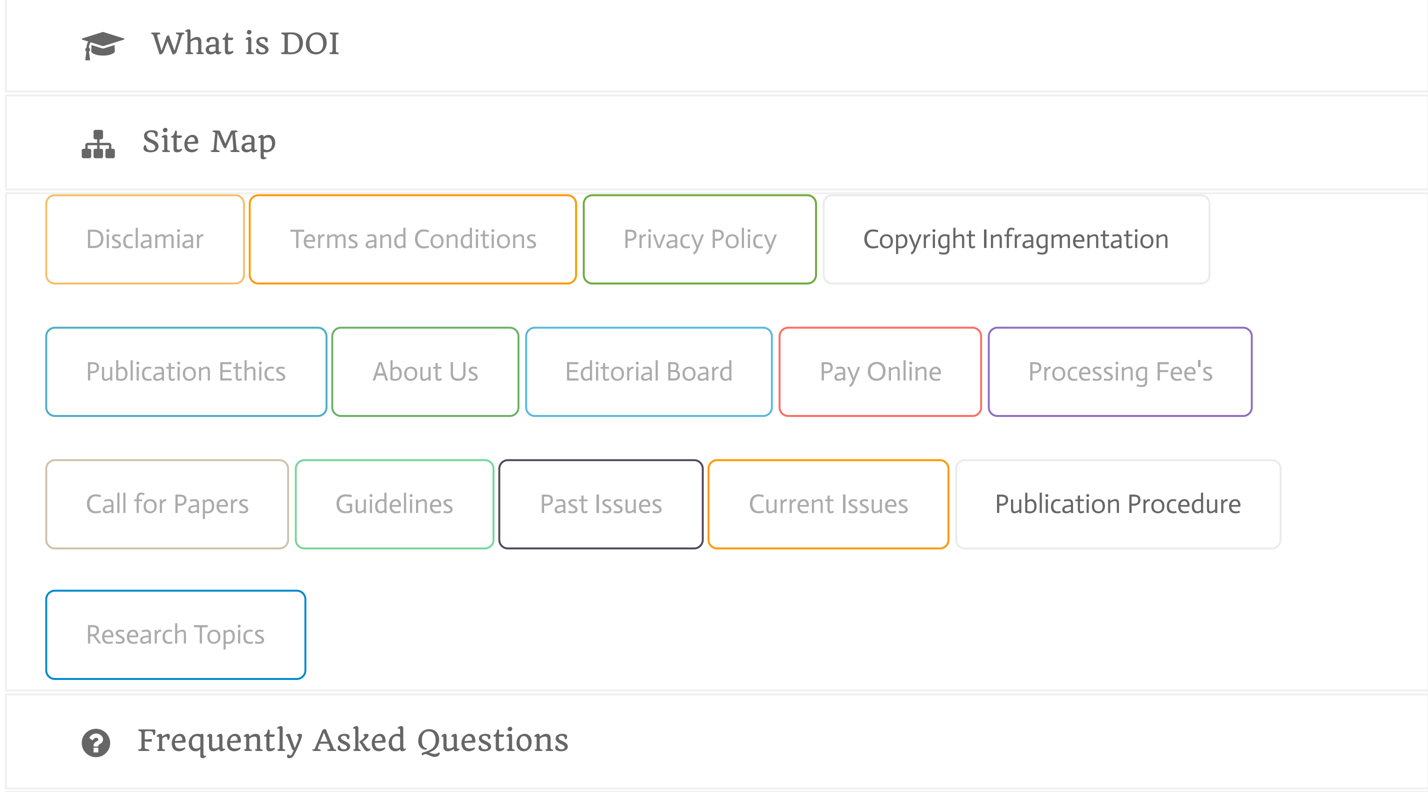

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Description

Disease Prediction System

1.Prof. Shikalgar A. A.,2Tanuja D. Supekar, 3Aishwarya A. Shinde, 4Arpita S. Phadtare,5Shradddha S. Potekar

Professor, Student, Department of Computer Science and Engineering

Yashoda Technical Campus Wadhe, Satara

Abstract
Health prediction is a predictive modeling application that predicts disease based on the user's symptoms as i nput to the system. The system evaluates the symptoms given by the user as input and outputs the result of t he disease. It can be given in the form of a virus entered here, and symptoms are given from the text format. The input in the text indicated by the placement of the checkbox with the symptoms. Depending on the input provided by the user, the output will only be produced as text for different objects.

Disease prediction is made using the classification decision tree based on the Random Forest algorithm for th e first type of input.This application is used for community health, biomedical needs, etc. where medical infor mation can be verified using large print data so it can be beneficial for users' health.

Keywords Machine Learning, Symptoms-based disease prediction, detector, Decision tree, Image processing, application, technology.

A Review of Decentralized Energy Management Strategies for Microgrids and

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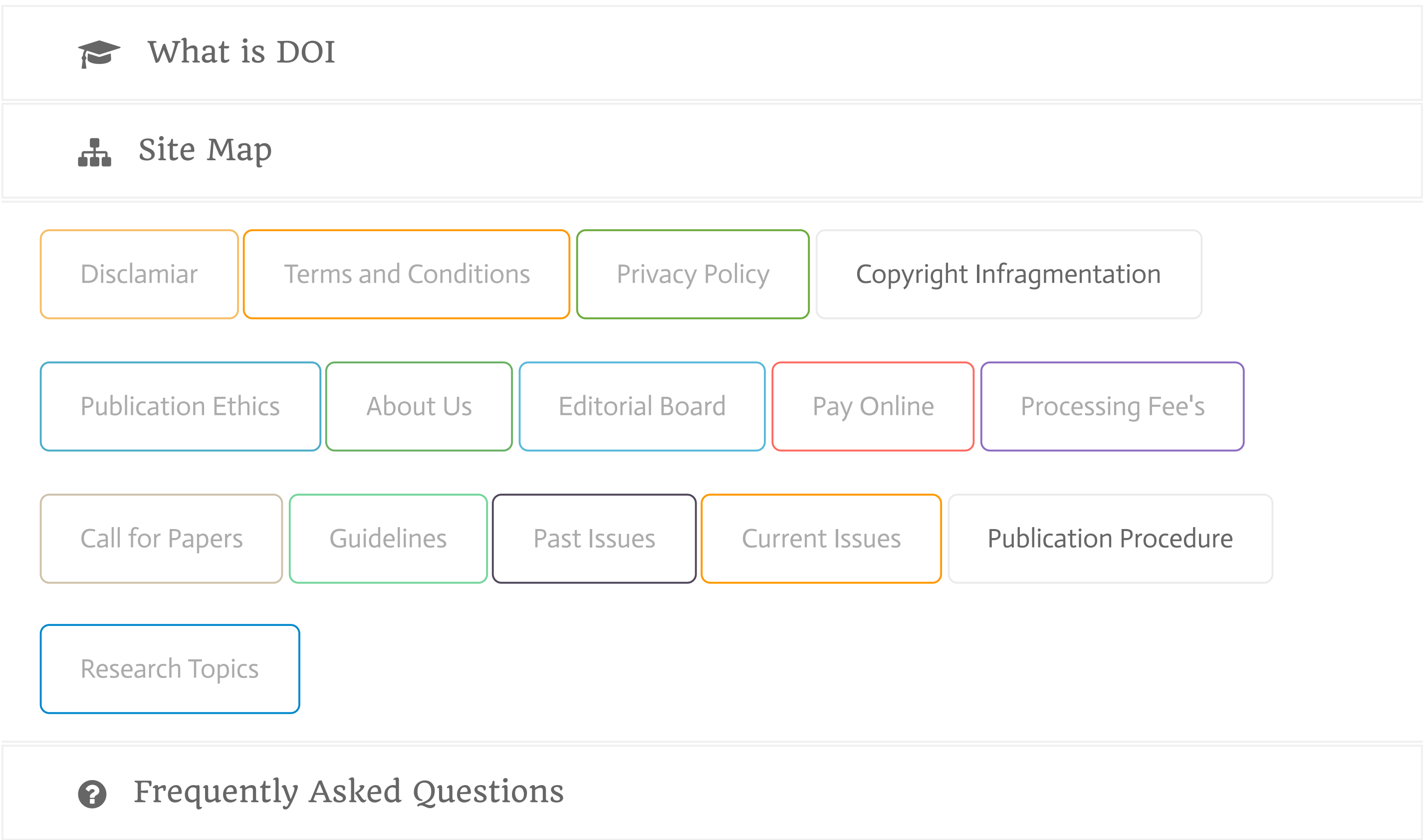
Optimization Approaches



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QUIZZLES: Test Your Skills and Become a Master

[1]Prof. Himgouri Tapase, [2]Miss. Neha Bobade, [3]Mr. Shrijeet Desai and [4]Miss. Shruti Kesarkar

Department of Computer Science and Engineering, YSPM's Yashoda Technical Campus, Satara-415001, Affiliated to DBATU University Lonere, Maharashtra, India.
Corresponding author:Prof. Himgouri Tapase
Email id:gouribarge.8@gmail.com

ABSTRACT

"Quizzless" is a collection of a number of different types of quizzes. Online Quiz system is a web based multiple-choice-question system; a system that can be used by lectures to evaluate students efficiently, effectively and perfectly. The purpose of the Online Quiz System is to save lecture's time since the answers are automatically marked. A user can access/play the entire quiz and can attempt any of the one. There will be a limited number of questions and for each correct answer the user will get a credit score. There are many quiz applications available currently on the internet. But there are few which provide better understanding between users and the application like, providing proper answers, etc. A system that is an online application, from which admin can easily manage Academic details, Quiz details, Student details. The developed system is a user-friendly quiz application. Which will contain: Numbers of quiz, Answers to every question to improve the knowledge level of users. To develop an application which will contain solutions to the above problems. By this application the user will come to know about his/her level and can learn additional knowledge. Also, by this application a user can expand his/her knowledge among the world.

KEYWORDS: Quizzless, Online Tests, Easy evaluation, Fast result

INTRODUCTION:

Nowadays, a lot of universities in our country and each of the Universities consist of up to two thousand students. In order to handle large numbers of students may cause a lot of problems, especially in managing the quiz manually. Currently, almost all universities in our country use the manual procedure to set up a quiz for students. The manual procedure means in every quiz, students must attend university to sit in the quiz at a specific time. After that, lectures will collect quiz papers. Sometimes, there are students who do not attend university for a reason and lecturers assume the student is absent for the quiz. This scenario is unfair for students who missed the quiz. So the suitable solution for this problem is by design a system that all student can sit in a quiz from any location

"Our aim was to develop an application for the users in which a user can attempt any number of quizzes related to his/her choice."

Quizzless is a software developed to conduct an Online Quiz based on time constraints. The Quiz System is accessed by entering the username and e-mail id which is added to the database. Quiz is started by displaying a definite number of questions with four options each based on the category chosen i.e., General Knowledge, Aptitude Test, Technical Test, Logical Test etc. Admin can also add another test. If the answer is correct, score is incremented by marks decided by admin and no negative marks for wrong answers. Final score will be displayed and updated in the database with username.The prime objective of "Quizzless" is to take a quiz of any individual through application. It is a system by which students can appear in a quiz from anywhere in the world where there is no interaction between paper and pen rather than interaction between computer and human being. Any college, school or educational institute can use this system for their organization to take quizzes. Today it is a more efficient and effective method of accessing distant students. It is automated marking, that is teachers do not need to check the answer script as they do in manual quiz. It provides a unique functionality. It saves valuable time for people. Students can increase their knowledge via giving this kind of quiz and prepare best for their upcoming future. The feedback form is also provided for students in case of complaint.

SCOPE:

The main objective of "Quizzless" is to facilitate a user-friendly environment for all users and reduce manual efforts. In past days the quiz was conducted manually but with further resolution of the technology we are able to generate the score and pose the queries automatically. The functional requirements include to create users that are going to participate in a quiz, automatic score and report generation and administrative tasks like add, delete, update for admin privilege users. In this application, all the permissions lie with the administrator i.e. Specifying the details of the quiz with checking the result will show to the user or not, addition of questions and answers, marks for each question, set timer for each quiz and generate a report with score for each quiz. The application Quizzless also conducts the exams very effortlessly. This application is really useful in prevention of cheating. It will lead to safe and secure data; it will have a high level of data integrity. In traditional mechanisms there was a lot of pressure on administration of everything. This application will minimize the burden of administration. The feedback system will also be provided for the purpose of enhancing educational knowledge. It can be used anywhere anytime. This application can be used in educational as well as in the corporate world. In the future in this application, we can implement signing in via QR code. Admin can upload excel files of questions to add questions into the exam. Also feedback after every test and progress report.

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LITERATURE REVIEW:

Quizzes can add insight and enhance students' abilities about the subject matter that is being learned. Quizzes can also stimulate students to learn. Said that the use of technology in the learning process will cause excitement because students interact with images, videos, and animations. This condition of pleasure is an important factor in learning effectiveness. Understanding the website is an internet facility that connects documents in a local or long-distance scope. Documents on the website are called web pages and links on the website allow users to move from one page to another (hypertext), both between pages stored on the same server and servers around the world. The idea draws motivation from the people who hesitate to visit the police station and personal belief of weak investigation and corruption and limited spreading of crime information. The usage of the internet is increasing in every sector, so people find it easier to register a complaint online rather than visiting the police station, it is secure and possible to hide their identity if they want.

This application helps to create a bridge between normal people and the police department to share information and evidence. It is helpful to track and monitor the criminals around the state and country and maintain a complete record of criminal information. It helps to search and access a large amount of data in less amount of time and provides a language-independent search over a large amount of data collected from different sources. It provides online assistance and general information and creates awareness among the people.

MODULES AND THEIR FUNCTIONALITIES:

There are two modules in this system:

1. Admin
2. Student

1. Admin Module:
    - Authentication Phase.
    - After Login, you can see the Total Number Of students, Tests, questions are there in system on Dashboard.
    - Can View, Update, Delete Student.
    - Can Also See Student Marks.
    - Can Add, View, Delete Tests/Exams.
    - Can Add Questions to Respective Tests With Options, Correct Answers, And Marks.
    - Can View And Delete Questions Too.

2. Student Module:
    - Authentication Phase.
    - Create account (No Approval Required By Admin, Can Login After Signup)
    - After Login, You Can See How Many Courses/Exams And Questions Are There In System On Dashboard.
    - Can Give Exams Any Time, There Is No Limit On Number Of Attempts.
    - Can View Marks Of Each Attempt Of Each Exam.
    - Question Pattern Is MCQ With 4 Options And 1 Correct Answer.

3. Exam Module:
    - There are a number of tests like technical, logical, aptitude, gk etc.

4. Result Module:
    - Each and every attempt will be stored with date, time and marks.

IMPLEMENTATION:

Admin page is used strictly by the administrator to set up his/her parameters so as to enable him/her to have total control over the system. Only the admin can search for student details which he/she wants to edit, delete or blacklist. The student module he/she is accessed by entering the username and e-mail id which is added to the database. Quiz is started by displaying a definite number of questions with four options each based on the category chosen i.e General Knowledge, Aptitude Test, Technical Test, Logical Test etc. he/she chooses one test, then a quiz is started displaying rules to read carefully then press start exam button. If the answer is correct, score is incremented by marks decided by admin and no negative marks for wrong answers. After completing the exam final score will be displayed and updated in the database with the username.

TESTING TECHNOLOGY:

Software testing technologies is a process which is used to measure the quality of software developed. It is also a process of uncovering errors in a program and makes it a feasible task. It is a useful process of executing programs with the intent of finding bugs. In order to prove that a piece of software works, the software must be tested to determine if the requirements of the application are met. There are several different types used throughout the development process. These are various types of testing. Some of which are mentioned below:

- Component Testing: Where each and every component related to the software project is tested. Component testing should focus on testing component interfaces.
- System Testing: The testing implemented on an overall software project after component integration is system testing. System testing tests the emergent behavior of a system.
- Acceptance Testing: It is performed after software installation in a user environment with data supplied by customers.

Following are the technologies are going to use:

1. Unit testing: It is a level of software testing where individual units or components of a software are tested. It is a process of testing individual components in isolation.
2. Integrated testing: It is a level of software testing where individual units are combined and tested to verify if they are working properly.
3. Beta testing: It is one of the types of user acceptance testing. Where a release of the software is made available to a large group of users to allow them to experiment and to raise problems that they discover with system developers.

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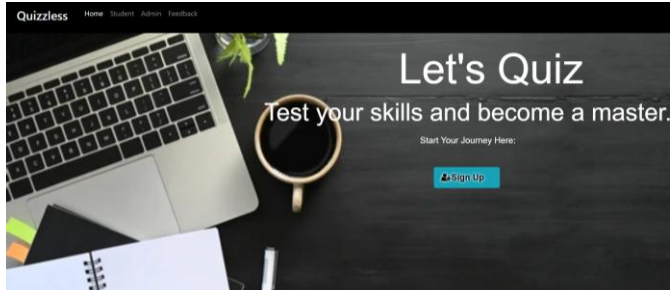
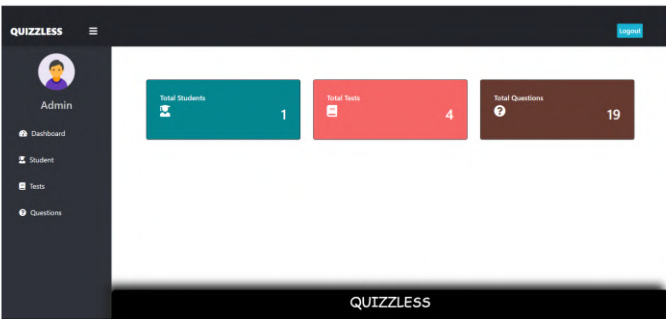
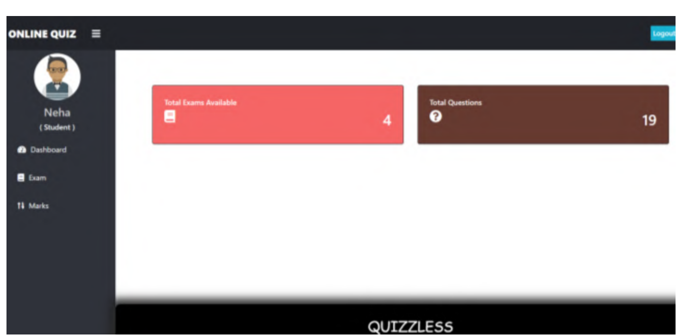
INPUT DESIGN:



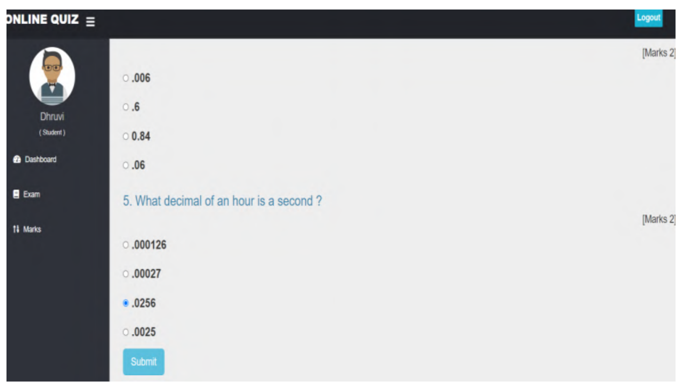
Home quizzless web pages



Administrator’s quizzless web page.



Student’s quizzless web page.



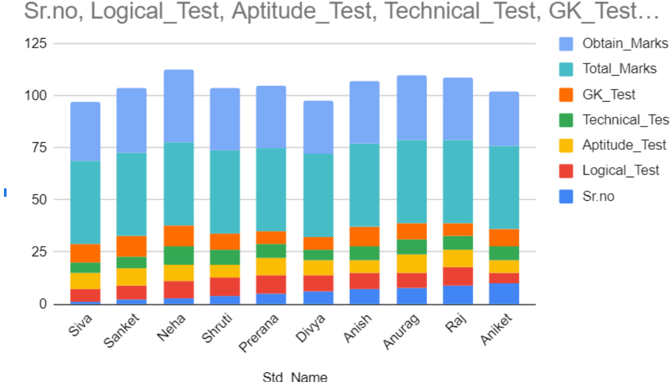
Student’s exam web page.

RESULT:

Student Result_Table

Sr.no	Std_Name	Logical_Test	Aptitude_Test	Technical_Test	GK_Test	Total_Marks	Obtain_Marks
1	Siva	6	8	5	9	40	28
2	Sanket	7	8	6	10	40	31
3	Neha	8	8	9	10	40	35
4	Shruti	9	6	7	8	40	30
5	Prerana	9	8	7	6	40	30
6	Divya	8	7	5	6	40	26
7	Anish	8	6	7	9	40	30
8	Anurag	7	9	7	8	40	31
9	Raj	9	8	7	6	40	30
10	Aniket	5	6	7	8	40	26

Student Result_Graph



ADVANTAGES:

The system is very simple in design and to implement. It has got following features:

- Reduce the damages to the machines.
- Minimum time needed for the various processes.
- Greater efficiency.
- Better service.
- User-friendliness and interaction.
- Minimum time required.
- It saves more time.
- It saves the student's money.
- It saves paper.

LIMITATIONS:

- Time limit is not given.
- Adding questions is kind a difficult.
- Admin can add any number of questions to any test, But while adding test, admin provide question number.

FUTURE SCOPE:

- The future scope of this project is very broad in terms of gaining knowledge and sharing knowledge among the world.
- Time limit system will be implemented.
- Students can login via QR code.
- Adding questions will be easier.
- This can be used in educational institutions as well as in the corporate world.
- Can be used anywhere any time as it is a web based application, (user location doesn’t matter).

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CONCLUSION:

Quizzless application provides facility to attempt quizzes anywhere and anytime. It saves time since the user does need to wait for the result. So, students/users cannot wait for the result. All students/ users get extra knowledge and skills. Administrator has a privilege to put as many questions in a given Quiz in application. Users can register, log-in to attempt Quiz. If a user forgets their password, then he/ she can reset the password with the help of Forgot Password option. There is an instruction page for students to get information about quizzes like number of questions, given time etc. After that students get the result of the quiz. So, students don't have to wait for the result. Also, the admin is responsible to add, delete and update questions in the system. Also, he can view the results of all students. This project proved good for me as it provided practical knowledge of programming in python and MySQL server and also about all handling procedures related to the Quiz System. It also provides knowledge about the latest technology used in developing application and server technology that will be in great demand in future.

This will provide better opportunities and guidance in future in developing projects independently. This Quiz Application can be efficiently used by any students and educational platforms. The project will be made as per as the given specification. The system will be as user-friendly as possible.

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Description

NFT Music Marketplace

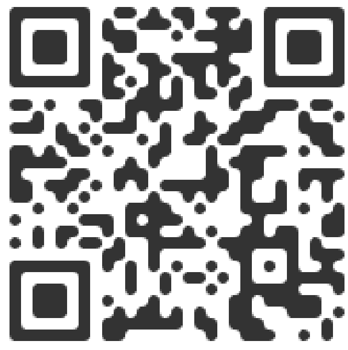
Prof. Tapase.H.O, Bhoite Kshitij Sujit, Pawar Amardeep Vivek, Soni Ayush Lalchand, Momin Huzefa Ajamuddin.

Professor, Student, Department of Computer Science and Engineering, Yashoda Technical Campus Wadhe, Satara.

ABSTRACT

The project Music NFT marketplace is a decentralized music platform created by our group. The purpose of the blockchain is to allow the collection and distribution of digital data, but the data cannot to be modified. People interacting on this platform will be able to buy, sell, play the music deployed on a IPFS (decentralized network). Users interact with the NFT Music through their Metamask wallet by paying some cryptocurrency.

KEY WORD: Blockchain, Music NFT, Smart Contracts.



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SCANNING & DETECTION OF VIRUS USING CRYPTOGRAPHIC HASH FUNCTION

Prof. Himgouri O.Tapase[*1], Bhavika N. Oswal[*2], Kajal N. Katkar[*3], Rutuja A. More[*4], Ruturaj R. Jadhav[*5]

[*1,2,3,4,5]Department Of Computer Science & Engineering, YSPM's Yashoda Technical Campus, Satara, Affiliated To DBATU University Lonere, Maharashtra, India.

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ABSTRACT

This paper based on the detection and scanning the virus by using the cryptographic hash function. In our system different algorithms are used. By using this system we are scan the files, folders, drives and also scan external devices such as pen-drive, mobiles etc.& detect the virus. This system is used to protect our devices from the malware or virus. Generally this system provide security for our devices from virus and save the important data, information. This desktop Application maintain privacy of data & provide security. Now days all the people use computers for various activities : online gaming, shopping, emails, study at this time the risk of virus infection in the computer is rising, people loss their data but they don't understand how computer get infected by virus. so to maintain user data safe, secure devices this system is developed.

Keywords: Hash Function, Virus, Security, Computer, Algorithms, Mobiles.

I. INTRODUCTION

Today we live in digital era where all aspect of lives depend on the network, computer and other electronic devices and software application. The technique of protecting internet connected system such as computers, servers, mobile device, electronic system from virus called cybersecurity. We can divide this is in the two words "cyber" and other "Security".

Now a days there is various growth in the smart phones, laptops, computers and applications, the amount of the virus damage the user privacy, crash their data. In today's world mobiles, computers are used to shopping, online gaming, office purpose, social network etc. So there is need to protect our data & devices. Keeping devices secure is the important task, so secure devices from the virus.

Securing devices is important thing or big challenge because the virus are increasing day by day. Virus is computer program that damage ,destroy ,crash devices and the users personal data. Also virus is a computer program that can copy itself and infect a computer without permission and knowledge of user. The original virus may modify the copies,copies modifies themselves, as occurs in virus & this system detect virus after the computer download & runs the executable. There are various ways to virus entered into the computers and make harmful to the devices.

There are two common method of anti-virus system that scan and detect the virus. first is "virus signature definition" & second "heuristic algorithm". Today we are in digital era where most of things are depends on the network, computer, applications. All the infrastructure such as government, financial institutions, banking sector, healthcare section are connected to the internet, some of information is important such as financial data, government data, personal data, so there is most of the chances of unauthorized access & losses of data because of infected by virus so "virus scanning and detection system is developed".

This is the paper of virus scanning and detection. In this system first of all user can register. After registration user can login with the id and password. login page block the unauthorized user. In my profile page shows all information related to a user. So user can get his id and username of registration. User interact using user interface. This interface has many options. Using that options user will perform tasks. Scan drive module Using scan drive option user will scan specific drive. In that option all files will scan which drive user has selected.

There are two modules one is shows scanned files and another shows infected files. there is one option that is Main Page. Using that option user can go back to user interface. In manual scan user can select the particular file or folder which he wants to scan. Which include all types of file formats. This manual scan option shows

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selected particular file. So user can access that file. If we select any file for the scanning then this shows the particular files generates the unique value called as checksum value. After that scan the selected file and display the result. This shows result of scanned particular file. If newly generated **MD5** checksum value match with stored value then it shows MD5 checksum value is found in virus dictionary otherwise it shows **MD5 checksum** value is not found in virus dictionary. If viruses not detected using above all options then user can select second filter option to scan the computer system. User can scan the all drive. This show scanning process of all drives using command prompt. MD5 value generation show that if user wants to generate MD5 checksum value then user select particular file and generate checksum value. After that the select MD5 generator This shows that user select the particular file that he wants to generate checksum value. Then MD5 value can be generated of selected file by the user. Also it generate checksum value in virus dictionary and show it.

II. METHODOLOGY

ALGORITHM USED:

1. MD5:

It stands for Messages digest Algorithm. It is cryptographic hash function algorithm in that this algorithm take message as input of any certain length means not fixed length and produce output into fixed length output i.e 16 bytes length. It is advanced security purpose. This algorithm is improvement in MD4 algorithm. The digest size of MD5 is 128 bits.

Working of MD5:

STEPS:

1. Append Padding Bits
2. Append Length Bits
3. Initialize MD buffer.

2. SHA-256:

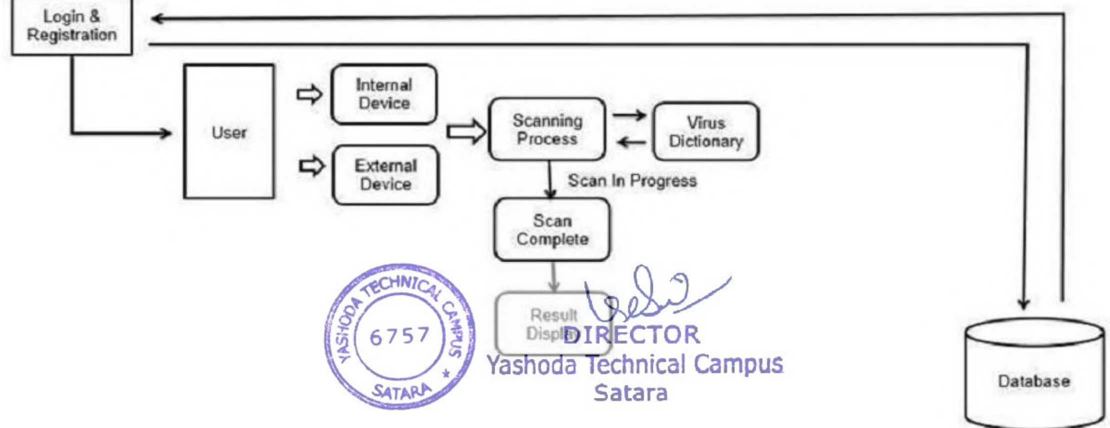
SHA 256, stands for secure hash algorithm 256, it is used for the message, file verification. It is part of the family which is SHA-2. It takes data as input is 256-bit key and convert into the new string of fixed length, this string of the character and digits called as Hash value & which is also in 256 bit in size.

SHA 256 follows following steps:

1. Data is converted into binary code in the form of 0 & 1.
2. Binary data is divided into the certain block 512 bits.
3. The message is divided into small block & each blocks are 32 bits.
4. Sixty-four rounds of compression function are performed.
5. New hash value are created from output of previous operation.
6. Final 256-bit hash value produced.

MODELING

The system is divided into various module and each module functionality is different.



Module 1. Registration :

The new user initially registers to our system. In Registration form the user fills the basic details. This details are saved in database. In registration form system the page takes solely the valid details.

Module 2. Login :

Here, user has to put his login credentials which he has created during opening his profile in system by fill the registration form. When user registration is completed user moveto login page. This page blocks the unauthorized users.

Module 3. User Interface:

User interact using user interface. This interface has many options. Using that optionsuser will perform tasks.

Module 5. Scan Drive:

Using scan drive option user will scan specific drive. In that option all files will scan which drive user has selected. There are two modules one is shows scanned files and another shows infected files. there is one option that is Main Page. Using that option user can go back to user interface.

Module 6. Manual Scan:

In manual scan user can select the particular file or folder which he wants toscan which include all types of file formats.

Module 7. Second Filter:

If viruses not detected using above all options then user can select second filter option to scan the computer system. User can scan the all drive. This shows scanning process of all drives using command prompt.

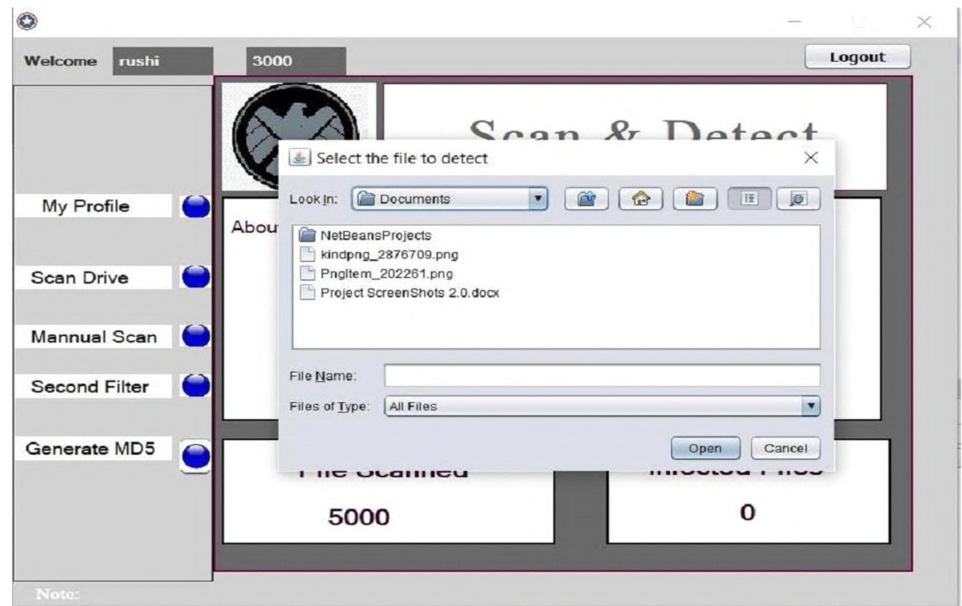
Module 8. MD5 Value Generation:

This shows that if user wants to generate MD5 check sum value then user selectparticular file and generate checksum value.

III. RESULT

Manual Scan:

In this user can select the file which they want to scan. Also it include all the types of file format.



Generate Checksum Value:

This shows the file generate unique value called as checksum value. It is the combination of the string & number.

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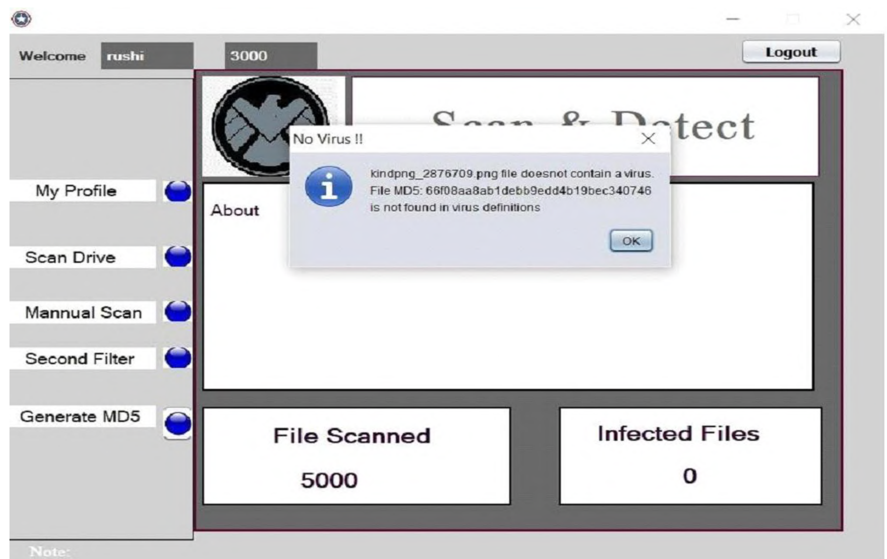
Scanned Files:

This shows the result of the scanned particular file. If newly generated MD5 checksum value is match stored checksum value then MD5 checksum value found in virus dictionary otherwise value not found in virus dictionary.



IV. CONCLUSION

Viruses are very destructive programs that can be devastating to companies and individuals. Upon completion of this papert students should be able to have an understanding of the following: what viruses are, how they get into a computer, how viruses can be avoided, how you get rid of viruses, and the best type of software used to prevent viruses. Basically this is virus scanning and detection system so by using this system we can protect our data fromthe virus. Main conclusion of this project is to secure and save the important data, files, folders, and external devices.

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we would like to thank my parents who patiently helped me as i went through my work and helped to modify and eliminate some of the irrelevant or un-necessary stuffs.

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Criminal Detection Through Facial Recognition – A Research Paper

Prof.Nalawade Suraj Project Guide(CSE) YSPM Satara
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Gaikwad Anuj Ajit Student(BTech CSE) YSPM Satara

Abstract – Criminal Face Detection project aims to build an automated Criminal Face Detection system by levering the human ability to recall minute facial details. Identification of criminals at the scene of a crime can be achieved in many ways like fingerprinting, DNA matching or eye witness accounts. Out of these methods eye witness accounts are preferred because it stands scrutiny in court and it is a cost effective method. It is possible that witnesses to a crime have seen the criminal though in most cases it may not be possible to completely see the face of the perpetrator. The Criminal Face Detection System will be built of an existing criminal database. Input would be provided in the form of sketch or an image and matched against the existing database and results would be provided. Criminal record generally contains personal information about particular person along with photograph. To identify any Criminal we need some identification regarding person, which are given by eyewitness. The human face is a complicated multidimensional visual model and hence it is very difficult to develop a computational model for recognizing it. The paper presents a methodology for recognizing the human face based on the features derived from the image. The proposed methodology is implemented in two stages. The first stage detects the human face in an image using viola Jones algorithm. In the next stage the detected face in the image is recognized using a fusion of principle.
Keywords – Privacy, face detection, Algorithms, Investigation.

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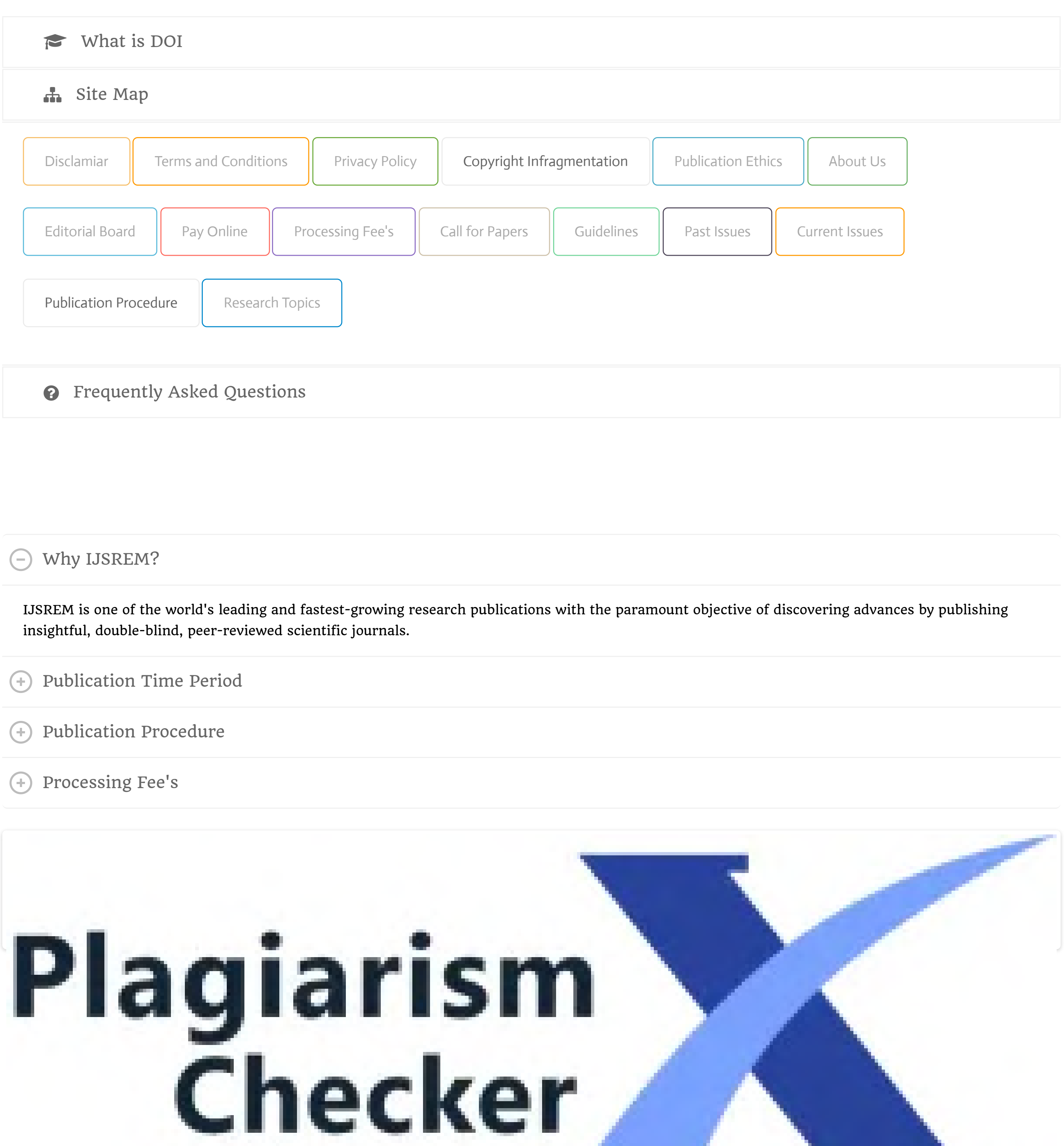

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20	Smartphone User Behaviour Predication Using AI	Dr. S V Balshetwar	International Journal for Research in Applied Science & Engineering Technology	https://doi.org/10.22214/ijraset.2022.40906
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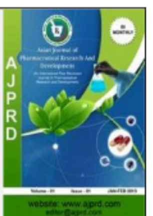

Research Article

Acute Toxicity Study and Anti-Nociceptive Activity of Ethanol Extract of *Aesculus Indica* Seeds on Experimental Animal Models

Priyanka R. More*, Atish B. Velhal, Vitthal J. Chaware, Vivek Kumar K. Redasani

YSPM's Yashoda Technical Campus, Wadhe, Satara, MS, India.

ABSTRACT

Aesculus indica, widely known as the horse chestnut tree, has long been used as antiangiogenic, antibacterial, antidiabetic, antiviral and antifungal. Ttraditionally it has been used as medicine for the treatment of skin diseases, rheumatism and different pain conditions. The current study was undertaken to investigate possible effects of ethanol extract of seeds of plant in experimentally produced pain in animals because there were no scientific publications on the use of *Aesculus indica* seeds for anti-nociceptive activity. Preliminary phytochemical screening revealed the presence of flavonoids, tannins, carbohydrates, Saponins, and phenolic substances in the extract. The OECD guideline 423 was followed for acute toxicity testing.At a dose of 2000 mg/kg, the extract was confirmed to be safe. The anti-nociceptive effect of three distinct dose levels of extract (100, 200, and 300 mg/kg) was tested in Swiss albino mice using a hot plate, tail immersion test, and acetic acid induced writhing. Extract had strong anti-nociceptive efficacy ($P < 0.001$) in a hot plate test. The extract significantly increased the tail withdrawal reaction in the tail immersion test ($P < 0.001$).The extract considerably reduced the number of writhes in the acetic acid writhing test ($P < 0.001$). The findings indicate that the extract has substantial anti-nociceptive effect.

Key Words-Anti-nociceptive, Acute toxicity, *Aesculus indica*, Phytochemical screening.

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***Address for Correspondence:**

Priyanka R. More ,YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara, MS, India.

INTRODUCTION

Pain is more than just a vexing sensation; it is a complicated sensory mechanism. Pain is caused by the activation of nociceptors at peripheral nerve terminals in response to tissue injury, which results in the release of a range of chemicals that change the local environment and cause pain.[1] Many pathological aches progress due to inflammatory responses in the central and peripheral nerve systems.[2] When nociceptive signals from the peripheral nerve system to the brain are regulated, pain is usually felt. In reaction to tissue injury or stimuli, a variety of chemicals such as histamine, bradykinin, and prostaglandins are released, resulting in nociception.[3]Also implicated in nociception are endogenous opioid and cannabinoid receptors[1]. Treatment of pain in chronic inflammatory disorders such as rheumatoid arthritis is a major issue for clinicians and the general public, as chronic use of current synthetic medications has dangerous side effects that cannot be ruled out. This needs the creation of a new nociceptive agent that is both safe and effective in eradicating or minimising unwanted effects. Herbal medications are used in several developing countries, despite the fact that they are not documented in science. Traditional folk remedies are used by about 80% of the population in some developing nations.[4]Pain has traditionally been treated using a variety of herbal medicines. Phytoconstituents have been scientifically verified for anti-nociceptive action.[5-8]The seeds of *Aesculus indica* (Family –Sapindaceae) arerich in saponins most specifically aescin.[9] Aescin has been reported to have anti-nociceptive activity via inhibition of oxidative stress and inhibition of prostaglandins.[10] Additionally with saponins*Aesculus indica*also contains flavonoids and tannins, it may show anti-nociceptive activity. Moreover Anti-nociceptive activity forextract of *Aesculus indica* leaves has been reported in the literature.[11] Hence the

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present research work was aimed to evaluate anti-nociceptive activity for ethanol extract of *Aesculus indica* seeds in experimental animal models.

MATERIALS AND METHODS

Drugs and reagents

Aspirin was purchased from USV Pharma, Mumbai, India and pentazocine was purchased from Themis medicare Ltd., Haridwar, India. All chemicals and reagents used for the experiments were of analytical grades.

Collection and authentication of plant material

Seeds of the *Aesculus indica* were procured from Royal Rifco Company, Shrinagar, India.After collection seeds were cleaned, washed to remove any dirt, dust and foreign particles. Botanical identity of plant specimen was authenticated by Dr. S. A. Mohite,Head, Department of Botany, Lal Bahadur Shastri College, Satara (MS), India.A voucher specimen of theseeds has been deposited in the department for future reference.Theseeds were coarsely powdered and further utilized for preparation ofethanol extract.

Preparation of ethanol extract

The ethanol extraction of seeds of *Aesculus indica* was carried out by soxhlet apparatus. The seeds were crushed and ground to powder and placed into extractor. The ethanol was poured on powder with three cycles. After that extraction process was started and continued till appearance of solvent in syphon tube turns brown to clear. Then brown colored solvent mixture from round bottom flask was collected and evaporated with the help of rotary evaporator to get a solid residue. The residue was placed in a vacuum desiccator and was further used for the experiments.

Prelminary phytochemical screening:

Prepared ethanol extract was subjected to preliminary phytochemical screening for presence of Alkaloids, Glycosides, Carbohydrates, Phenolic compounds, Flavonoids, Saponins, Reducing sugars.[12,13]

Experimental animals

Swiss albino mice (18–25 g) were provided byYashoda Technical Campus, Faculty of Pharmacy, Wadhe. Satara. Animals were housed and maintained according to standard guideline and procedures with animal facility at relative humidity 75 ± 5% temperature 22 ± 2° C, and a 12 h light/dark cycle. Animals were provided with standard diet and purified water ad libitum. Mice were allowed to acclimatize to the environment for seven days before start of the animal study. The experimental protocol was approved by Institutional Animal Ethics Committee. All the animal experimental procedures were performed according to the National Institutes of Health (NIH) guidelines on handling of experimental animals.

Acute oral toxicity study

The acute oral toxicity was performed as per the Organization for economic co-operation and development (OECD) guideline 423.[14]Acute toxicity study was performed in Swiss albino mice. The animals were grouped with three numbers in each. Ethanol extract of *Aesculus indica* seeds was given to animals with starting dose 300mg/kg in 0.1% CMC for first group.

According to observations of first group, study was carried out further on next group with dose 2000 mg/kg. From obtained results it was clear that no death as well as no toxicological signs in animals so, for confirmation of safety of extract study was repeated with dose 2000mg/kg on third group. After administration of extract, animals were observed carefully for first 30 min. and periodically for 24 h with special attention during first four hours. Animals were further observed daily for subsequent 14 days. Effects such as changes in skin fur, eyes and mucous membranes were observed daily. Also the circulatory, autonomic, respiratory, and central nervous systems, behaviour pattern and somatomotor activities were observed during study. Animals were further observed for salivation, diarrhea, tremors, lethargy, convulsions, sleep, and coma. The parameters like body weight, food, and water intake were checked periodically every two days.

Anti-nociceptive activity:

The anti-nociceptive activity of ethanol extract was tested using different animal models namely hot plate, acetic acid induced writhing and tail immersion test. Doses of extract were selected based on results of acute toxicity study.

Healthy Swiss albino mice (18–22 g) were used for the study. Animals were divided into five groups of six in each. Group I was control and received 0.1% CMC, group II was received standard drug, group III, IV and V were received ethanol extract of *Aesculus indica* seeds with low, medium and high dose by oral route.

Evaluation parameters

Tests were performed on same animals after 14 days washing period.

Hot plate test

Analgesic activity in mice was executed according to the method described previously.[15,16] The hot plate analgesiometer (IITC, USA) was used to determine the analgesic activity of ethanol extract of *Aesculus indica* seeds. Animals were divided into five groups of six in each. Group I received vehicle i.e 0.1% CMC by oral route, group II received standard drug pentazocine 17 mg/kg by intraperitoneal route, group III, IV and V were received ethanol extract of *Aesculus indica* seeds 100mg/kg, 200mg/kg and 300mg/kg respectively in 0.1% CMC solution by oral route.

Animals were placed on hot plate at different time points (0, 15, 30, 45, 60, 90 and 120 min) after administration of standard drug pentazocin, extract and vehicle, time require for first response (flickering or licking of hind paw or jumping) has been measured. Hot plate was maintained at 55 ± 0.5 °C and cutoff time fixed for 15 sec. to avoid tissue damage.

Acetic acid induced writhing test

The acetic acid induced writhing test was performed as described previously using 0.1 ml of 0.6% v/v acetic acid solution in normal saline.[17,18] Swiss albino mice (18–22 g) of either sex were divided into five groups of six in each.

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Group I received vehicle i.e 0.1% CMC by oral route, group II received standard drug Aspirin 30 mg/kg by oral route, group III, IV and V received ethanol extract of *Aesculus indica* seeds 100mg/kg, 200mg/kg and 300mg/kg respectively in 0.1% CMC solution by oral route. Thirty minutes after administration of standard drug Aspirin and test extracts, 0.1 ml of 0.6% acetic acid were administer via intra-peritoneal route. The number of writhing will be counted for 20 minutes after administration of acetic acid. The percentage inhibition of writhing has been calculated.

percentage inhibition of writhing = (C – T / T) x 100

Where C- Average number of writhes in control group.

T- Average number of writhes in test group.

Tail immersion test

The tail immersion test was carried out in Swiss albino mice (18–22 g) according to method described by previous researchers.[19,20] Animals were divided into five groups of six in each. Group I received vehicle i.e 0.1% CMC by oral route, group II received standard drug pentazocine 17 mg/kg by intraperitoneal route, group III, IV and V were received ethanol extract of *Aesculus indica* seeds 100mg/kg, 200mg/kg and 300mg/kg respectively in 0.1% CMC solution by oral route. Animals were adapted for restrainer 30 min. before study leaving the tail hanging out freely. After administration of standard drug pentazocine, extract and vehicle, the tail immersed in hot water (Temperature 55 ± 0.5° C) and the reaction time require for removal of tail has been recorded as response. The cutoff time for tail exposure to hot water fixed to 15 s. The response was recorded at 0, 15,30,60,90,120 and 180 minutes after administration of dose.

Statistical Analysis

The data presented as a mean ± SD (Standard Deviation). Two way ANalysis Of VAriance (ANOVA) was used to make comparisons between the treated groups. The level of statistical significance was set at P < 0.001.

RESULTS

Preliminary phytochemical analysis:

Table 1.Shows the findings of qualitative analysis of *Aesculus indica* seeds extract. According to the obtained resultscarbohydrates, saponins, tannins, flavonoids were found to be present in extract.Alkaloids, glycosides, amino acids, steroids and terpenoids were found to be absent.

Table 1. Qualitative analysis of the phytochemicals inseeds extracts of *Aesculus indica.*

Sr. No.	Test for Phytoconstituents	Present/Absent
1.	**Alkaloids**	
	Mayer's Test	Absent
	Dragendroff's Test	Absent
	Wagner's Test	Absent
	Hager's Test	Absent
2.	**Glycosides**	
	Keller Killiani's test (Cardiac Glycosides)	Absent
	Borntrager's test (Anthraquinone Glycosides)	Absent
3.	**Carbohydrates**	
	Molish Test	Present
	Fehling test (reducing sugar)	Present
4.	**Steroids**	
	Salkowski's Test	Absent
5.	**Flavonoids**	
	Lead Acetate Test	Present
	Sodium Hydroxide Test	Present
6.	**Saponins**	
	Foam Test	Present
7.	**Tannins and phenolic compounds**	
	Ferric Chloride Test	Present
	Lead Acetate Test	Present
	Dilute Nitric Acid Test	Present
	Dilute Iodine Solution Test	Present
	Acetic Acid Solution test	Present
8.	**Proteins**	
	Biuret test	Absent
9.	**Amino acids**	
	Ninhydrin Test	Absent

Acute toxicity study

The acute toxicity study began with a 300mg/kg starting dose. During a 14-day observation period, oral administration of a 300 mg/kg dosage of ethanol extract of *Aesculus indica* seeds caused no significant toxicity. From above results it is clear that given dose was safe and hence further study was performed by administering 2000mg/kg dose of extract to next group of animals. There were no indicators of toxicity and mortality [Table 2.], as well as the animals' morphological characteristics and general appearance did not change. There was no salivation, diarrhoea, tremors, convulsions, lethargy or unusual behavior observed during study in treatment group. When compared to control group mice, extract-treated animals did not demonstrate any significant changes in body weight, food and water intake [Table 4.]. For further confirmation of results effect was checked by giving same dose (2000mg/kg) to another group of three animals and results

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were repeatedly same. Table 3, shows the parameters measured before and after the test extract of *Aesculus indica* seeds. According to results even at the highest dosage of 2000mg/kg body weight of the test animal, all parameters were normal. The oral LD_{50}could be over 2000mg/kg body weight. As a result, greater dose testing of the extracts may not be necessary, and the extracts were practically non-toxic.

Table 2. Effect of *Aesculus indica* seeds extract for sign of toxicity and mortality (n = 3).

Group	Treatment	Sign of toxicity (ST/NB)	Mortality (D/S)
Normal Control	Vehicle	0/3	0/3
Aqueous extract	2000 mg/kg	0/3	0/3
Alcoholic extract	2000 mg/kg	0/3	0/3

ST = Sign of toxicity, NB = Normal behaviour, D = Died, S = Survived.

Table 3. Effects of A.i. seeds extractat dose 2000mg/kg on morphological characteristics and general appearance in mice (n=3).

Sr. No.	Response	Before	After
1.	Alertness	Normal	Normal
2.	Touch response	Normal	Normal
3.	Torch response	Normal	Normal
4.	salivation,	Normal	Normal
5.	Diarrhoea	Absent	Absent
6.	Tremors	Absent	Absent
7.	Convulsions	Absent	Absent
8.	Lethargy	Absent	Absent
9.	Skin fur	Normal	Normal
10.	Pinna reflux	Normal	Normal
11.	Corneal reflux	Present	Present
12.	Pupils	Normal	Normal
13.	Lacrimation	Normal	Normal
14.	Gripping strength	Normal	Normal
15.	Urination	Normal	Normal
16.	Hyper activity	Absent	Absent

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Table 4. Effect of extract of *Aesculus indica* seeds extract 2000mg/kg on body weight, food intake and water intake of mice (n = 3).

Day	Normal control			Test group		
	Body weight (g)	**Food intake (g)**	**Water Intake (ml)**	**Body weight (g)**	**Food intake (g)**	**Water intake (ml)**
0	19.20 ± 1.15	6.16 ± 0.51	6.86 ± 0.25	19.60 ± 0.85	6.06 ± 0.23	6.20 ± 0.17
2	19.23 ± 1.05	6.20 ± 0.36	6.93 ± 0.73	19.73 ± 0.90	6.20 ± 0.20	6.66 ± 0.40
4	19.56 ± 0.95	6.06 ± 0.61	7.13 ± 0.72	19.80 ± 0.80	5.90 ± 0.17	6.90 ± 0.10
6	19.76 ± 0.76	6.20 ± 0.36	7.20 ± 0.65	20.03 ± 0.85	5.83 ± 0.05	7.03 ± 0.56
8	19.90 ± 0.75	6.26 ± 0.25	6.93 ± 0.51	20.20 ± 0.90	6.16 ± 0.15	7.03 ±0.11
10	20.20 ± 0.65	6.46 ± 0.41	7.30 ± 0.45	20.50 ± 0.75	6.33 ± 0.15	7.03 ±0.23
12	20.46 ± 0.65	6.46 ± 0.57	7.03 ± 0.11	20.56 ± 0.86	6.03 ± 0.20	6.76 ±0.25
14	20.66 ± 0.60	6.46 ± 0.41	7.00 ± 0.17	20.93 ± 0.75	6.13 ± 0.30	6.80 ±0.30

All data is expressed as Mean ± SD (n = 3).

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Antinociceptive Activity:

Hot plate test:

The effect of ethanol extract of *Aesculus indica*seeds is represented in Table 5. Extract significantly delayed the response dose dependently at doses 100, 200 and 300 mg/kg between 15 and 120 min after administration of extract when compared to control group. At dose 300 mg/kg showed maximum response at 45 min with response time 8.80 ± 0.14 ($p < 0.001$) when compared with control group (3.05 ± 0.18). The standard drug pentazocine showed maximum response at 30 min with reaction time 10.03 ± 0.17 ($p < 0.001$) when compared with normal control animals (3.2 ± 0.30).

Table 5: Effect of ethanol extract of *Aesculus indica* seeds in hot plate method.

Groups	Reaction time in seconds						
	Basal	**15 min.**	**30 min.**	**45 min.**	**60 min.**	**90 min.**	**120 min.**
Normal control	2.88 ± 0.14	3.06 ± 0.19	3.2 ± 0.25	3.05 ± 0.18	2.96 ± 0.08	3.03 ± 0.10	3.00 ± 0.15
Pentazocine 17 mg/kg i.p.	2.93 ± 0.26***	8.70 ± 0.08***	10.03 ± 0.16***	9.93 ± 0.17***	7.80 ± 0.14***	5.78 ± 0.07***	4.76 ± 0.12***
Ai extract 100mg/kg p.o.	2.86 ± 0.10***	4.01 ± 0.14***	6.18 ± 0.17***	6.58 ± 0.17***	4.83 ± 0.17***	3.88 ± 0.14***	3.25 ± 0.15
Ai extract 200mg/kg p.o.	2.68 ± 0.14***	5.31 ± 0.14***	6.68 ± 0.19***	7.21 ± 0.19***	5.03 ± 0.12***	4.38 ± 0.28***	3.53 ± 0.32***
Ai extract 300mg/kg p.o.	2.98 ± 0.14***	6.11 ± 0.25***	7.36 ± 0.16***	8.80 ± 0.14***	7.66 ± 0.20***	5.08 ± 0.22***	4.63 ± 0.08***

All data is expressed as Mean ± SD (n = 6).

*** $p < 0.001$when compared with control.

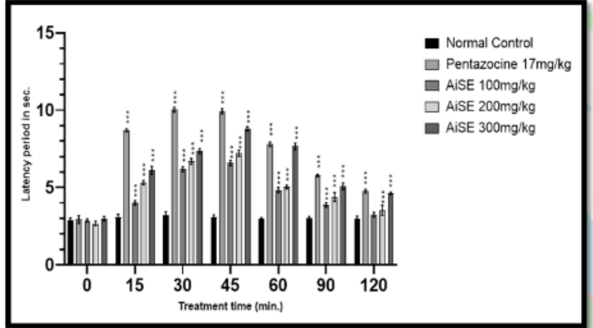

Figure 1: Effect of the *Aesculus indica* seeds extract and pentazocine on the latency time of mice in hot plate model. Values shown are mean ± SD, *** $p < 0.001$when compared with control, n=6.

Tail immersion test

The table 6. Shows effect of ethanol extract of *Aesculus indica* on time required for tail withdrawal response in mice. Extract at all doses significantly increased response time in comparison with control group. The highest dose (300 mg/kg) of extracts (9.63 ± 0.23; $p < 0.001$) showed maximum response at 1.5 h which was considerable response in comparison with pentazocine (17 mg/kg) (12.55 ± 0.25).

Table 6: Effect of ethanol extract of *Aesculus indica* seeds in tail immersion test.

Groups	Time for tail withdrawal response (Seconds)						
	Basal	**15 min.**	**30 min.**	**60 min.**	**90 min.**	**120 min.**	**180 min.**
Normal control	2.11 ± 0.23	2.06 ± 0.10	2.25 ± 0.13	2.18 ± 0.17	2.21 ± 0.18***	2.30 ± 0.16	2.25 ± 0.19
Pentazocine 17 mg/kg i.p.	2.15 ± 0.17***	4.08 ± 0.16***	7.05 ± 0.16***	10.10 ± 0.19***	12.55 ± 0.16***	10.08 ± 0.16***	8.21 ± 0.13***
Ai extract 100mg/kg p.o.	2.18 ± 0.11***	3.05 ± 0.12***	4.60 ± 0.26***	6.13 ± 0.13***	7.30 ± 0.14***	4.26 ± 0.18***	3.25 ± 0.13***
Ai extract 200mg/kg p.o.	2.21 ± 0.11***	3.26 ± 0.19***	5.25 ± 0.22***	7.35 ± 0.13***	8.36 ± 0.11***	6.16 ± 0.08***	4.16 ± 0.17***
Ai extract 300mg/kg p.o.	2.11 ± 0.13***	3.53 ± 0.12***	6.21 ± 0.11***	8.26 ± 0.10***	9.63 ± 0.16***	7.53 ± 0.17***	5.25 ± 0.10***

All data is expressed as Mean ± SD (n = 6).

*** $p < 0.001$when compared with control.

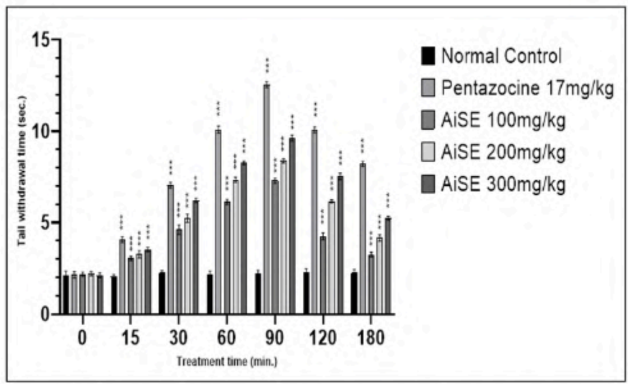

Figure 2: Effect of the *Aesculus indica* seeds extract and pentazocine on the reaction time of mice in Tail immersion model. Values shown are mean ± SD,*** $p < 0.001$when compared with control, n = 6.

Acetic acid induced writhing test

Table 7.Shows the effect ofethanol extract of *Aesculus indica* seeds on the number of writhing in mice. When compared to the control group, extract significantly reduced the number of writhes at doses of 100 mg/kg, 200 mg/kg, and 300 mg/kg. In comparison with control group (44.66 ± 1.44) the extract at 300 mg/kg showed the greatest suppression of writhes (27.16 ± 1.16, $p < 0.001$). When compared to the normal control, the aspirin (30 mg/kg) inhibited writhing by 87.41 percent, whereas the extract at 300 mg/kg inhibited writhing by 64.43 percent.

Table 7: Effect of ethanol extract of *Aesculus indica* seeds in writhing test.

Groups	No. of writhing	% Inhibition
Normal control	44.66 ± 0.81	-
Aspirin 30 mg/kg p.o.	23.83 ± 0.75***	87.41
AiSE 100mg/kg p.o.	37.60 ± 0.81***	18.77
AiSE 200mg/kg p.o.	32.5 ± 0.54***	37.41
AiSE 300mg/kg p.o.	27.16 ± 0.75***	64.43

All data is expressed as Mean ± SD (n = 6).

*** $p < 0.001$when compared with control.

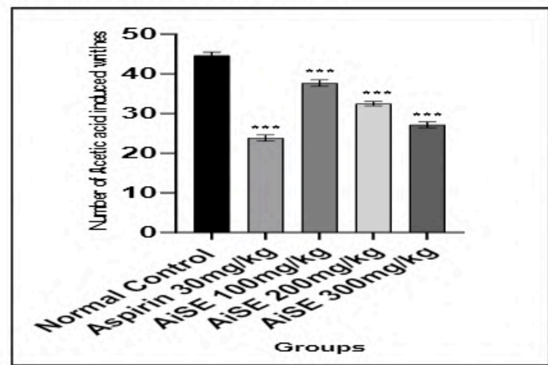

Figure 3. Inhibitory efect of the *Aesculus indica* seeds extract and aspirin on the acetic acid-induced writhes in mice, Values shown are mean ± SD, *** $p < 0.001$when compared with control, n = 6.

DISCUSSION

For centuries, medicinal plants have been utilised to treat human illnesses. The active compounds are mostly responsible for the crude drug's biological activity. However, evidence-based scientific research on the biological activity and toxicity of medicinal herbs are limited. The findings of this study revealed that the *Aesculus indica* seeds extract contained carbohydrates, saponins, tannins, and flavonoids [Tab.1]. These secondary metabolites produce a definite physiological action on the human body.[21-27] Toxicity data aids in determining the maximum dose of a substance that can be safely utilised in animals and humans. There were no reports on the toxicity of *Aesculus indica* seeds extract. As a result, the current investigation began with acute toxicity of the extract at a dose of 300 mg/kg and then progressed to a dose of 2000 mg/kg after obtaining satisfactory results. During 14 days of treatment with a single dose of *Aesculus indica*, there was no death. There was no substantial change in body weight, food, or drink intake, safety of extract.No symptoms of toxicity were noticed at the limit dose throughout the investigation, indicating that it was well tolerated. The findings indicated that the extract is safe to use.

The central mechanism of analgesic action of the extract was evaluated using hot plate and tail immersion techniques.To measure central anti-nociceptive activity, the tail immersion and hot plate tests are standard and very sensitive assays. With this nociception models, the extract showed substantial action. It is well known that centrally acting analgesics raise the pain threshold of mice when they are exposed to heat.[28] These tests are important in determining whether or not a heat induced nociception is present and whether or not narcotics are involved.[29] The hot plate test is very sensitive test to determine central anti-nociceptive activity. It involve neuronal signaling pathways to respond thermal stimuli.Supraspinal reflex is elicited by hot plate method. The substances, which increases the reaction time against heat stimulus, act centraly to mimic pain.[30]*Aesculus indica* (100, 200 and 300 mg/Kg) prolonged the latency period in hot plate model and tail withdrawing time as that produced by pentazocine, a standard analgesic drug indicating the centraly mediated anti-nociceptive activity.

Both tests use neural signalling pathways to respond to heat stimuli and are linked to central activity.[31] As a result, these tests were used to distinguish between central and peripheral analgesics. The hot plate involves higher brain functions and indicates a supra-spinally structured reaction to thermal pain stimuli, whereas the tail immersion test involves spinal motor reflexes to thermal nociceptive stimuli. The effect of central analgesics like opioids is mediated via regulation of spinal (μ2, κ1, δ2) and supraspinal (μ1, κ3, δ1, σ2) receptors.[32]

To test peripheral nociception acetic acid-induced writhing paradigm was used. Acetic acid causes the hind limbs to extend, the back to arch, and the abdominal muscles to contract in response to peripheral nociception.[33,34] TNF-, interleukins, and other inflammatory mediators such as histamine, bradykinin, serotonin, substance P and prostaglandins are released when acetic acid is given intraperitoneally.[35,36]The nociceptors in the dorsal horn of the central nervous system are activated by cytokines and inflammatory mediators, which then activate inflammatory

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pathways, resulting in pain feeling.37,38 These mediators stimulate chemosensitve nociception, developing abdominal constrictions. Such pain sensations are inhibited by non-steroidal anti-inflammatory drugs (NSAIDs), which exhibit antinociceptive effect by inhibiton of prostaglandin synthesis.[39,40]Ethanol extract*of Aesculus indica* seeds reduced the acetic acid-induced writhing, similar to caused that of aspirin revealing the antinociceptic effect of *Aesculus indica*, possibly through the inhibition of peripheral pain mediated pathways.

The presence of saponins, flavonoids and tanins in *Aesculus indica* may account for the anti-nociceptive effect found, as these phytochemicals are renowned for their analgesic effect, while the involvement of additional constituents present in the plant cannot be overlooked.

CONCLUSION

The current study found that at the dose levels tested as per the acute toxicity studies, the ethanol extract of *Aesculus indica* seeds has considerable dose dependent antinociceptive effects in laboratory animals. The findings show that antinociceptive activity of *Aesculus indica* is mediated by two analgesic pathways: peripheral and central.

The tail immersion and hot plate tests are linked with central activity and involve neuronal signaling pathways to respond thermal stimuli. Tail immersion test involves spinal motor reflexes to thermal nociceptive stimuli, whereas the hot plate involves higher brain functions and represents supra-spinally organized response to thermal pain stimuli. The peripheral nociception induced by acetic acid was inhibited by extract. Possible mechanism peripheral analgesic effect may likely due to cytokine inhibition and other inflammatory mediators.

The presence of saponins, flavonoids and tannins in *Aesculus indica* seeds, along with the existence of other phytochemicals, may be responsible for antinociceptive effects. The findings appear to back up the plant's historic use in the treatment of several painful illnesses and also point to the presence of biologically active compounds. However more research is needed to isolate and characterize the bioactive components responsible for the anti-nociceptive effect.

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Review Article

Pharmaceutical and biotechnological applications of microsponges as novel nano technological drug delivery system

Shankar B. Kalbhare, Atish B. Velhal, Mandar J. Bhandwalkar, Rupali V. Jadhav, Akash S. Nalawade*

Department of Pharmaceutics, YSPM's, Yashoda Technical Campus, Satara, India 415003

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Abstract

Microsponges drug delivery system composed of porous microsphere. They are tiny sponges-like spherical particles with a larger porous surface. Morever they may enhance stability, reduce side effect and modify drug release favorably. Microsponges technology has many favorable characteristics, which make it a versatile drug delivery system. Microsponge system are based on microscopic, polymer-based microsphere that can suspend or entrap a wide variety of substance, and it can be incorporated into a formulated product such as a gel, cream, liquid or powder. The outer surface is typically porous, allowing a sustained floe of substance out of the sphere. Microsponges are designated to deliver a pharmaceutical active ingredient efficiently at the minimum dose and also to enhance stability, reduce side effect, and modify drug release.

Keywords: Controlled release, Healthcare system, Microsponges, Microsponges Delivery System

Introduction

One of the major challenges in pharmaceutical industry is to control the release of a drug at the specific organ in the body. Now days there are various systems are for targeting the delivery of a drug to a specific organ eg.trasdermal delivery system (Kalbhare et al., 2020). But the transdermal system are not proven for the delivery of the drugs which target the skin. For gastric cancer, there are no systems available which give local effect along with the controlled release of drug. Therefore it is a challenging area for the research work. Microsponges is a type of drug delivery system that enables controlled release and transport of active ingredients too the target organ.

The microsponge drug delivery system was invented by Won in 1987, and the first patent was assigned to Advanced Polymer System. This industry formulated different types of procedures which are applied in the cosmetic and pharmaceutical industry (Jadhav et al., 2013). Microsponge drug delivery systems are polymeric delivery systems composed of porous microspheres. They are small sponge like spherical structures that consist of a countless number of internally connected voids with a larger pores. It consists of non-collapsible structures. Moreover, they increase stability, reduce side effects and transform drug release. Because of the larger porous surface, the drug is released in specific manner. Microsponges have a number of favourable characteristics for targeted drug delivery. Microsponge drug delivery is based on polymeric microscopic spheres that can entrap and suspend wide variety of substances, and thenthey can be incorporated into a formulation such as a cream, gel, or powder. Microsponge drug delivery systemcan increase the efficacy, safety andproduct stability and improve the properties of the formulation in an effective manner (Jadhav et al., 2013; Kaity et al., 2010). Depending upon the size, pore length and pore volume, the microsponge drug delivery system releases the active ingredient.The release of the active ingredient depends on the rubbing, temperature and pH.Microsponges have the ability to absorb the load of polymers and active ingredients in the particles on their surface. Mostly microsponge systems are often used in the transdermal route (Mandavaet al., 2012; Barkai et al., 1990).

***Address for Corresponding Author:**
Akash Shivaji Nalawade
Department of Pharmaceutics,
YSPM's Yashoda Technical Campus,
Satara (India) 415003
Email: kirankal786@gmail.com

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The average size of the microsponges delivery system is in the range 5µm to 300µm in diameter size and a typical 25µm to 250000µm. The surface size of the microsponges varies 20 to 500 µm /g and pore volume range 0.1 to 0.3cm/g. This results in a large reservoir within each microsponge, which can be loaded with up to its own weight of active agent (Jadhav et al., 2013; Kaity et al., 2010; Embil et al., 1996). These pores can entrap large range of drug and other ingredients like emollients, fragrances, essential oils, sunscreens, anti-inflammatory agents. These formulations that can be applied into the targeted region and this entrapped material gets delivered to the skin and controls therelease of the drug.

Potential characters of microsponge

Microsponges are stable at pH range from 1-11 and at high temperatures Microsponges have good compatibility with different type of polymer and ingredients. They also have high entraptment efficiency up to 60-70%. The pore size of microsponges is small so that it prevents the penetration of bacteria. Microsponges does not require sterilization and the addition of preservatives. The system is cost effective and can be used for the long term treatment. The polymeric design of the microsponges is mainly utilized for the controlling the drug release for given period of time and also being used for targeting specific region.

Benefits of microsponges

The microsponges can enhance product performance and also extend the release of drug upto 12 hours. They reduce irritation, increase patient compliance and improve product elegance. Microsponges increase the physical, chemical, thermal stability of drugs and absorb the oil upto 6 times their weight. Because of flexibility of microsponges they can act as novel drug delivery systems. Microsponges are non-irritating, non-mutagenic, non-allergenic and non-toxi. Microsponges allow the incorporation of immiscible products.Microsponges can improve bioavailability of some drugs.

Method of preparation of microsponge

Preparation of Microsponges involves two steps which are liquid-liquid suspension polymerization and quasi emulsion solvent diffusion techniques or w/o/w emulsion technique that can be based on physico chemical properties of drug.

Liquid-liquid suspension polymerization technique

The porous polymeric microspheres can be prepared by liquid-liquid suspension polymerization method. In this method, immiscible polymers are first dissolved with active moities in a suitable solvent. The aqueous phase consist of additives like surfactant, suspending agents to form of suspension. The polymerization process is activated by increasing the temperature. Following this process, the development of reservoir system contributes to the formation of the porous structure. The solvent is then removed and the spherical porous structured microspheres are formed. These formed microspheres are known as microsponges (Burton et al., 2002; Chardeet al., 2013). If the drug is not suitable for the one step procedure mentioned above, then two-step process will be used for polymerization.

Quasi-emulsion solvent diffusion

By using quasi-emulsion solvent diffusion technique porous microsponges can be prepared.In this technique, the first phase is prepared by using eudragit and ethyl alcohol. Then, the active ingredient is added slowly in to the above phase and dissolved. The plasticizers like triethylcitrate (TEC) also added to impart plasticity. The internal phase is poured in the external phase which contains PVA and distilled water with continuous stirring for 2 hours. The product is washed and dried in a hot air oven at 40°C for 12 hr (Çomoğluet al., 2003; Kumariet al., 2016).

w/o/w solvent diffusion

Microsponges can be prepared by double emulsion technique using sodium chloride as a porogenic solution. After that the solution of ethyl cellulose, eudragit and active ingredient in ethanol and dichloromethane is prepared. 1% (w/v) Aqueous solution is prepared using sufficient amount of Span. An aqueous polyvinyl alcohol solution and mucoadhesive polymer is prepared separately and previously prepared w/o emulsion is added to it. This w/o/w emulsion was stirred for 8 hr. The microsponges were obtained by filtration and dried at 60°C in the hot air oven and stored in dessicator till use. A compliation of the advantages and disadvantages of various methodologies used for preparation of microsponges (Table 1).

Drug release mechanism of microsponges

The active moieties are entrapped in porous microspheres. The microsponges consist of an open structure so that active ingredients are free to move through vehicle until equilibrium is attained and vehicle becomes saturated. This results in flow of the drug from the microsponge to the skin.The microsponges are then retained on the surface of the skin and will continue the drug release to the skin and provide a prolonged release for longer period of time. If the drug is freely soluble in the vehicle, the final product will not provide the desired drug release. Therefore, while formulating microsponge, it is important to choose a vehicle which has minimum solubilizing power of the active moities.

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Table 1. A compilation of the advantages and disadvantages of various methodologies used for preparation of microsponges

Method	Advantages	Disadvantages
Liquid--liquid suspension polymerization	Can be suitably modified to one step or two step methods for drug loading	Probable entrapment of unreacted monomers and solvent traces. Non-uniform structure. Requires long time for the reaction of monomers. Requires two-step method for thermosensitive drugs that has low drug loading efficiency
Quasi-emulsion solvent diffusion	No monomer entrapment. Low solvent traces. High drug loading. No exposure of drug to ambient condition. Size of microsponges can be easily controlled by controlling the stirring. Spherical particles	Cannot be used for the loading of water-soluble drugs. Requires long time for the reaction of monomers. Drug should be soluble in a volatile water-soluble solvent
w/o/w emulsion solvent diffusion	Efficient for loading water-insoluble drugs. Can be used to entrap proteins and peptides	Uses water-insoluble surfactants that can be present as residues in the resultant microsponges
Addition of porogen	Highly porous structure with nicely distributed and interconnected pores	May cause disruption in structure
o/o emulsion solvent diffusion	No presence of surfactant traces in microsponges	Requires vigorous washing to remove the traces of organic solvents
Lyophilization	Easy quick reproducible results	May lead to cracking or shrinkage of microparticle
VOAG method	Results in microsponges can be used for targeted drug delivery	Requires reflux conditions
Ultrasound-assisted production	No traces of solvents. Quick and reproducible results	Irregular structure.
Electrohydrodynamic atomization method	Quick reproducible and results	Require cross-linking agents that may be potentially toxic. May lead to the binding of drug molecule to the monomer. Control of size of particle and pores requires expertise.

Microsponges can release the given amount of drug over a period of time. The release is influenced by physicochemical factors like pressure, temperature change and solubility etc.

They are described as follows:

Temperature change

At certain temperature, few entrapped active ingredients become viscous and suddenly get released from microsponges. Increase in temperature of specific region also increases the flow rate and release (Of et al., 2015).

Pressure

When pressure is applied microsponges release the active ingredients at the targeted region (Of et al., 2015).

Solubility

Microsponges are filled with water soluble excipients and they release the drug with water. The release of drug that can be activated by diffusion technique.

pH

pH dependent drug release can be achieved by modifying the coating on the microsponge.

Evaluation of microsponge

Particle size determination

Particle size determination of loaded microsponges can be calculated by optical microscopy. In this sample that can be placed on the slide and mechanical stage. In that mean particle size is calculated by measuring more than 300 particles. For cumulative % drug release of microsponges will be determined by plotting particle size versus time. In the final topical formulation, particles of sizes between 1nm and 25µm are required to be used.

Determination of Production yield and Loading efficiency.

Loading efficiency it can be measured by following equation:

Loading efficiency = Drug Content in Microsponge x 100

Production yield of microsponges can be calculated by the gravimetric method using following equation

Production yield =MMicro/MRM

In that,

Mmicro = Weight of formulated Microsponges.

MRM = Weigh of raw materials (Polymer and active ingredient)

All results can be calculated in the triplicates.

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Characterization of pore structure

In this case, the volume of pore and diameter are very important in controlling the strength and duration of the effect of the drug. Pore diameter also affects the release of drug from the microsponge system through the vehicle in which all ingredients are distributed. By using mercury intrusion porosimetry the pore size of microsponges, percent porosity, the surface area of pore, percent porosity filled, pore diameters, shape and morphology of the pores, void volume, bulk, and apparent density can be determined.

In-vitro release studies

It is done by using dissolution test apparatus USP XXIII with a modified basket having 5µm mesh size. The dissolution rate can be measured at 37°C and 150 rpm. The dissolution media are chosen in order to maintain sink conditions and solubility of active ingredients. Sample aliquots are withdrawn from the dissolution medium and analyzed by a suitable analytical method (UV spectrophotometer) at regular intervals of tim (Naga et al., 2019).

Polymer/ Monomer composition

Various parameters such as spheres size, polymer composition, and drug loading govern the drug release from microspheres. The composition of polymer can also influence the partition coefficient of the trapped active ingredient between the microsponge system and the vehicle, thereby directly affecting the release rate of trapped substance. Drug release of microsponges of the different polymer compositions can be studied by the plotting the graph in-between average % drug release versus time. Polymers exhibiting varying degrees of hydrophobicity or lipophilicity or electrical charges may be prepared to impart flexibility to the release of active ingredients. A variety of probable excipient combinations can be screened for their compatibility with drugs by studying their drug release profile (Barkaiet al., 1990).

Compatibility studies

Infra-red spectroscopy (IR) and thin-layer chromatography (TLC) is conducted to determine the compatibility of drug and excipient. Powder X-ray diffraction (XRD) and Differential scanning calorimetry (DSC) can determine the effect of polymerization or crystallanity of active ingredients. For DSC, approximately 5mg samples are weighed, sealed and heated at 15°C/min in nitrogen atmosphere (Shaha et al., 2010).

Resiliency

Viscoelastic properties (resiliency) of the microsponge system can be tailored to create beadlets which are soft, ion accordance with the requirements of the final formulation. It increases cross-linking and slows down the release rate. Therefore, tests for viscoelastic properties of microsponges are performed and optimized according to prerequisite, considering release a feature of time of interconnection (Shaha et al., 2010).

Physicochemical characterization of microsponges

Scanning electron microscopy

For morphology and surface characteristics, The sample is coated in the gold-palladium at room temperature under an argon atmosphere, and the microsponge surface characteristics can be analysed by scanning electron microscopy(SEM).

Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) is performed for the pure drug, polymer and the drug-polymer physical mixture and microsponge formulations. The samples are incorporated in potassium bromide discs and are evaluated using the FTIR spectrometer. The peaks corresponding to the characteristic bands of the drug must be preserved in the spectra of the microsponges to indicate that no chemical interaction or changes have occurred during the preparation of the formulations.

Powder X-ray diffraction (XRD)

Powder X-ray diffraction (XRD) can be performed for both pure drug, polymer and microsponge formulation to investigate the effect of polymerization on the crystallinity of the drug. The disappearance of the characteristic peaks of the drug in the formulation could indicate that the drug is dispersed at a molecular level in the polymer matrix (Kilmer et al., 2010).

Safety Considerations

- Allergenicity in guinea pigs.
- Eye irritation study performed in rabbits
- Mutagenicity in bacteria
- Oral toxicity study in rats.
- Skin irritation studies in rabbits (Kiliçarslanet al., 2003; Sato et al., 1988).

Limitations

The use of organic solvents poses threats like toxicity and flammability. Traces of residual monomers in the bottom-up approach can be toxic and dangerous to health. But these shortcomings can be overcome by proper quality control measures along with optimization and standardization of procedures e. g, post-manufacture washing (Mandava et al., 2012; Srivastavaet al., 2012).

Applications of microsponges

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This system can be used to increase the effect, safety, and quality of prescription as well as over the counter products. Microsponge drug delivery system can be used in various applications. Microsponges drug delivery is mainly applicable to oral and topical applications. Several patents have been reported using different types excipients due to which microsponges exhibit high loading capacity and sustained release ability. These studies offer the formulator a scope to formulate a wide variety of products. Over the counter (OTC) products that contain microsponge drug delivery system and various sunscreens, specialized rejuvenated products, and moisturizers (Kilmeret al., 2010). Some more application of microsponges give (Table 2). Some examples of microsponge drug delivery with their formulations and uses (Table 3).

Marketed formulations

Microsponges Drug delivery System is ideal for skin and personal care and cosmetic products. They can take up the excess of skin oil while retaining an elegant feel on the surface of the skin. This technology is presently employed in a considerable number of products sold by leading cosmetic and toiletry companies worldwide. These products include oil control lotions, moisturizers, conditioners, deodorants, lipsticks, skin cleansers, powders, makeup and eye shadows which offer various advantages. They are advantageous due to increased chemical and physical stability besides they show greater availability which reduces the skin irritation. The controlled release of the active ingredients and unique tactile qualities are other advantages of this system. Some marketed formulation of microsponges with their advantages (Table 4) with some filed patent related to the microsponges (Table 5).

Recent advances in microsponge drug delivery system

Various advances technology have been made by using different methods or techniques e.g. nanosponges, nanoferrosponges, mucoadhesivemicrosponges, and porous microbeads. β-CD nanosponges were also formulated and can be used for hydrophobic as well as hydrophilic drugs. This nanosponge can be developed by cross-linking the β-CD molecule by reacting the β-CD with diphenyl carbonate. Researchers also observed that incorporating cytotoxic substances in a nanosponge carrier system can increase the potency of the drug, these type of

Table 2. Applications of microspongesystem

Active agents	Applications
Anti-inflammatory e.g. hydrocortisone	Prolonged activity with lessened of skin allergic response and dermatoses.
Anti-dandruff e.g. zinc pyrithione, selenium sulfide	Reduced nasty odour with decreases irritation with increase in safety and efficacy.
Skin depigmenting agents e.g. hydroquinone	Improved stability against oxidation with increase in efficacy and aesthetic application.
Anti-fungals	Sustained release of active ingredients
Anti-acne e.g. Benzoyl peroxide	Reduced skin irritation and maintaining efficacy and sensitivity.
Antipruritics	Extended and improved activity.
Sunscreens	These are long lasting products having high efficacy with enhanced protection againstUv rays, and sunburns, sun related injuries at high concentration and with low irritation and sensitivity.

Table 3. Examples of microsponge drug delivery with their formulations

Microsponge Delivery Systems	Drug	Clinical Use
Gels	TerbinafineHCl	Anti-fungal
	Hydroxyzine HCl	Urticaria and atopic dermatitis
	Acyclovir	Viral infections
	Fluconazole	Inflammation
	Benzoyl peroxide	Anti-Acne Treatment
Lotions	Benzoyl peroxide	Anti-Acne Treatment
Creams	Hydroquinone and Retinol	Melanoma
Tablets	Indomethacin	Inflammation
	Paracetamol	Anti-pyretic
	Chlorpheniramine maleate	Hay Fever
	Ketoprofen	Musculoskeletal pain
	Paracetamol	Colon targeting
Implants	Poly (DL-lactic-co-glycolic acid)	Skin tissue engineering
Grafts	Poly (lactic-co glycolic acid)	Cardiovascular surgery
Injection	Basic fibroblast growth facto	Growth factor

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Table 4. Marketed formulations of microsponges

Product name	Manufacturer	Advantages
Carac Cream	Dermik Laboratories, Inc. Berwyn , PA 19312 USA	Carac Cream contains 0.5% fluorouracil; it includes 0.35% incorporated in a porous microsphere consisted of methyl methacrylate / glycol dimethacrylate cross-polymer and dimethicone. Carac is a once-a-day topical application . For the treatment of actinic keratosis caused by over- exposure to the sun.
Retin-A-Micro	Ortho-McNeil Pharmaceutical, Inc.	Retin-A-Micro contains 0.1% and 0.04% tretinoin entrapped into a porous microsphere consisted of methyl methacrylatedimethacrylate cross-polymer to enable inclusion of the active ingredient, tretinoin, in an aqueous gel. Used for the topical treatment of acne vulgaris.
Salicylic Peel 20 & 30	Biophora	Salicylic acid 20% has been used in to it.Microspongesystem used for stimulat the skin for for faster results. Itimprove pigmentation, fine lines and acne. Salicylic acid passes easily through the pores.
Line Eliminator Dual Retinol Facial Treatment.	Avon	Retinol (Vitamin A) in MicrospongesDrug Delivery Systeem, for wrinkle-fighting action it release by two ways like immediate and timely release of drug. It clearly reduses appearance of lines and wrinkles.
Micro Peel Plus /Acne Peel	Biomedic	It stimulates the cell turnover so the application of salicylic acid in the form of microcrystals,These microcrystals target the specific areas of the skin. It is the chemical peels releases in to the skin of all dead cells while doing no damage to the skin.
Retinol cream, Retinol 15 Night cream	Biomedic, Sothys	Night cream. Microsponge technology it conatains pure retinol, Vitamin A. It diminishment of fine lines and wrinkles,
Lactrex™ Moisturizing Cream	SDR Pharmaceuticals, Inc., Andover , NJ , U.S.A. 07821	Natural humectant is used for soften and help to moisturizing the dryskin, cracked skin. It also contains 12% lactic acid as a neutral ammonium salt, ammonium lactate,water and glycerine.
Oil free matte block spf20	Dermalogica	Oil-free sunscreen protect the skin from damaging UV-rays while controlling the oil production and givesyou a healthy matte finish. That can be formulated with microsponge technology, Oil free matte block absorbs oil and prevents the shine without any powder esidue.
Sportscream RS and XS	Embil Pharmaceutical Co. Ltd.	Topicalprepareation It gives analgesic-anti-inflammatory and counterirritant actives for the management of musculoskeletal conditions.
Oil Control Lotion	Fountain Cosmetics	Microsponges that can absorb the oil from surface of skin, Eliminatethe shine for hours with this feature-weight lotion, formulated with oil-absorbing Microsponge technology. It can be mainly use for the Acne-Prone, oily skin conditions.s

carriers can be used mainly for the targeting the cancerous cells (Hu et al., 2007). Nanosponge, a novel approach constitutes the self- performing carriers having better penetration to the targeted site due to the external magnetic triggers which enforce the carriers to penetrate to the deeper tissues. Thereafter, the removal of magnetic material from the particles is effected leaving a porous system (Cavalli et al., 2006). The improved characteristics of porous microspheres, led to the development of a process to produce the porous microbeads. This method (High internal phase emulsion, HIPE) consisted of the monomer containing continuous oil phase, a cross-linking agent and aqueous internal phase (Çomoğlu et al., 2007) They also observed increased stability of RNA and the relatively effective encapsulation process of siRNA. This approach may lead to novel therapeutic routes for siRNA delivery (Lee et al., 2012)

Future prospects

Microsponge drug delivery system holds a promising opportunity in various pharmaceutical applications and industry in the coming future as it has unique properties like enhanced the product performance and elegancy, extended the release of active moieties, improved drug release

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Table 5. Patents Filed Related to Microsponges

Patent no	Inventors	Publication Date
US4690825	Won, Richard	1987
US4863856	Dean RC Jr et al.	1989
US5292512	Schaefer et al	1989
US5135740	Katz et al.	1992
US5679374	Fanchon; Chantal et al	1994
US5316774	Eury, Robert P et al.	1994
US5725869	Lo; Ray J. R.	1996
US6395300	Straub et al.	1999
US6211250	Tomlinson et al	2001
US20030232091	Shefer et al.	2002
US20040247632	Cattaneo, Maurizio	2004
US20050271702	Wright, Steven G et al.	2005
WO2008097429A1	Franklin Sadler Love	2007

profile, reduced irritation, improved physical, chemical, and thermal stability which makes it flexible to develop novel formulations. The real challenge in the future is the development of the delivery system for oral peptide delivery by changing ratios of polymers. The use of bioerodible and biodegradable polymers for drug delivery enables it for the safe delivery of the active material. These porous systems have also been studied for drug delivery through a pulmonary route, which shows that these systems can show effective drug release even in the scarce of the dissolution fluid. Therefore, colon is an effective site for targetted drug release. Development of carrriers for alternative drug administration routes like parenteral and pulmonary route is necsessary. These particles can also be used as cell culture media and thus can also be employed for stem cell culture and cellular regeneration in the body. These carrier systems have also found their application in cosmetics due to their elegance,. These developments enabled researchers to utilize them for various purposes. These novelties in the formulation also a new way for drug delivery (Srivastava et al., 2012)

Conclusion

With the demand for innovative and highly efficient Pharmaceutical as well as Cosmetic products, the market holds considerable potential for Microsponge technology and the versatility they offer. Since the researchers have found the new and creative way to deliver actives moieties, they can realize that the full capability of these materials providing safety and stability. It also reduces side effects of the active moieties, enhances multi-functionality and also increases active ingredient compatibility with the excipients. Microsponge delivery system would be a winning and innovative strategy for future, in the Pharmaceutical and Cosmetic industry. Microsponges have a distinct advantage over the conventional topical dosage forms for the treatment of topical diseases; it is a new strategy or one of a kind of technology for the controlled release of agents. It is advantageous over other products by because it is non-mutagenic, non-toxic & non-irritant. Thus the microsponge drug delivery system has got a lot of potential and is an emerging field which is essential to be explored for research in future.

Authors contribution

All the authors have contributed to the preparation and editing of this systematic review article.

Conflict of interest

The authors declare that they have no conflict of interest.

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REVIEW ARTICLE

Role of Autodock vina in PyRx Molecular Docking

Rutuja P. Pawar*, Sachin H. Rohane
Department of Pharmacy, Yashoda Technical Campus, Satara, 415003.
*Corresponding Author E-mail: **rutujapawar5000@gmail.com**

ABSTRACT:
Molecular docking has been widely employed as a fast and inexpensive technique in past decades, both in academic and industrial setting. In current situation molecular docking software is very useful. It's a need of society. That's why this review is focused on docking. In that Autodock Vena by pyrx version is very useful software for literature. New approaches continue to be developed and value of published work grows at a rapid pace. In resend developments there will be increase in accuracy, time limite, advances in computing power to eventually accomplish the full potential of the area. This review precents the overview of the method and attempt to summerise recent developments regarding four main aspects of molecular docking approaches: bench marking set, Advances in consensus method, recent applications using, use of machine learning, algorithms in docking. This autodock vena software gives more information related to molecular docking by litareture survey.

KEYWORDS: Molecular Docking, Drug Discovery, Autodock vina by PyRx software.

INTRODUCTION:
Doking is on the front line of computational biology and drug discovery the explosion of structural and chemical information in recent years has rendered this use the computation approaches to discover developed and analyzed and similar biologically active molecules the computer aided drug discovery leads to virtual screen, energy calculations, ADME models and drug interactions this helps in scientists in minimizing the synthetic and biological testing.

Autodock vina in PyRx software is most prefferable software in the molecular docking. This software is important. The molecular docking approach can be used to model the interaction between small molecule and protein at the automic level allow as to characterize the behavior of small molecules in the binding site of target proteins as well as elucidate fundamental biochemical process. In drug discovery, protein –ligand or protein – protein. Docking plays an important role in predicting the orientation of the ligand. The ligand is searched in a six dimentional rotational or translational space to fit in the binding site.[1-3]

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REVIEW OF ARTICLES:
Vina design philosophyis not to require the user to understand its implementation. Autodock is suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug conditions bind to recepter of known 3D structure. Autodock vina does not require choosing atom types and pre-calculating grid maps for them. Docking using autodock version 4.0 of PyRx software. PyRx is an open source software to perform virtual screening it is a combination of sevral software.

PyRx includes a docking wizard. Specific aspects for using PyRx as well as consideration for data preparation docking and data analysis also describes. Drug discovery is attractive research area that enables application of cutting edges biomedical research to improve health of man people by active components of natural origin have been under enormous investigation as potential studies that were performed by using PyRx docking tool through autodock vina software M. Venkateshan and Etal used autodock vina PyRx software to check inhibition activity of Azaphenantherena derivatives on or over SARS COV-2. This software to provid the guidance about inhibition activity of Azaphenantherena.[4]

Maryam Fatima et al used PyRx software to check bioactivity and docking of Synthesized ligand. the compound can serve as promising lead for the

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development of new antifungal agents this software is used to check antifungal acivity this compound can serves as promising leads for the development of new antifungal agent.[5]

Usman Abdulfatai et al used to autodock vina verion 4.0 of PyRx software to check activity relationship study of anti-convelsant activity of aminobenzothiazole of their quantitative structure. This software is useful to provide guidance about activity of anti-convalsant.[6]

Usman Abdulfatai et al used autodock vina of PyRx virtual screening software to, molecular docking and quantitative structure activity relationship studies were carried out and 37 anticonvalsant compounds to develop a robust model for the prediction of anticonvalsant activities against gama aminobutyric acid aminotransferase this softare is useful to analyses of a few aminotransferase inhibitor activity.[7]

Ritika Srivastava et al used PyRx software to check a alkylated benzimidazol: designed, Synthesis, Docking, DFT analysis ADMET property activity against HIV and YFV. Series of Alkylated Benzimidazol derivatives was synthesized and screened for their anti-HIV, anti-UFV and broad-spectrum antivirus properties. The software useful to show excellent inhibitory property against the yellow fever virus with.[8]

Muhammad Baba Muh'd et al used PyRx software to study of anti ulcer activity of quinozolinone derivatives of quantitative structure activity relationship by using this software is useful to provide anti ulcer activity. this anti ulcer agents exhibiting good action against the receptor (H/K Atpase).[9]

Titilayo Omolara Johnson et al used autodock vina PyRx software to a ulcerative colitis is an implamation of the colon that can progress colorectal cancer if left untreated no medication completely cures ulcerative colitis and natural products are source of alternative approaches the anti imflamatory potential of phyllanthus nivosus leaf as a natural remady and as a source of new drugs against ulcerative colitis is validated.[10]

Mohammad Abdul Mumit et al used PyRx to study on vibrational and electronicspectra analysis of benzyl-3-N-(2,4,5-trimethoxyphenylmethylene) hydrazine. The absorption, distribution, metabolism, excreation and toxicity investigation predicted that tne compound has good drug like character.[11]

Mohamed Elbadawi et al used PyRx to virtual drugs screening revealing an oxofluorenyl benzamyd and bromonaphthalene sulfonamide hydrobenzoic acid compounds induced apoptosis in a dose dependant manner as analyzed by flow cytomery this two compounds binds to HDAC6 an inhibites its function and exerts cytotoxic activity by apoptosis induction this software used to HDAC6 inhibit.[12]

Shola Elijah Adenji et al used PyRx software to investigating and evaluating some active compounds as potent anti-tubercular agent against MTB CYP121 recepter this. This whole docking result against MTB CYP121 receptor provide a valuable approach for structure based design.[13]

Rina Herowati et al used to PyRx software to studies of chemical constituents of tinos spara cortifolia on glycogen phosphorylase. These software used to give the activity of glycogen phosphorylase in the liver and widely used in the treatment of diabetis mellitus.[14]

Abhay Jaiprakash Gandhi et al used to PyRx software to study the drug for management of SARS-COV2 with ayurvedic perspective along with silico study. The molecular docking and grid were generated this PyRx software of autodock.[15]

Nanda Kumar Yellapu et al used to PyRx software to study the modeling, molecular docking, probing catalytic binding mode of acetyl-CoA malate synthase G in brucella melitensis. The core domain pocket of MSC catalytic residue. these ligand leads could be the best prospective inhibitors to treat brucellosis. These software is useful for activity.[16]

Sabitu Babatunde Olasupo et al used PyRx software is the antidepressant properties in inhibition of serotonin transporter has been considered to be a good target for the treatment of mood disorders. the phenyl piperidine derivatives as inhibitors of serotonin transporter cheminformatics and molecular docking. this software is used to check the antidepressant activity.[17]

Aliyu Wappah Mahmud et al used Autodock vina PyRx software to the quantitative structure activity relationships provides a model that link biological activities of compound to their chemical structures and molecular docking study reveal the interathction between drug and it target enzyme. In this software to check the activity of antiplasmodium hybrid compound.[18]

Adedirin Oluwaseye et al used autodock vina in PyRx software to check on anticonvulsant activity of isoxazole and thiazole derivatives active in animal model. This software useful to provide the information about active in subcutaneous pentylenetetrazole in animal model.[19]

Aliya Nur Hasanah et al used Autodock vina in PyRx software is useful to extraction of atenolol from spiked

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REVIEW ARTICLE

A Role of Herbal Drug as an Immunity Booster during Covid-19 Pandemic

Dhanashri Aware*, Sachin Rohane
Faculty of Pharmacy, Yashoda Technical Campus, Satara 415015, India.
*Corresponding Author E-mail: **awaredhanashri109@gmail.com**

ABSTRACT:
As the world scrambles to find a cure for COVID-19, Medical research network around the world is trying to find out treatment against the novel corona virus infection. In this pandemic condition there is a need for herbal remedies to boost the innate and acquired immunity to fight against corona virus. There are other certain ways to boost the "Immune System" such as active lifestyle, healthy diet, physical exercise, relaxation and sound sleep. Home remedies can be played a vital role as immunity modulator. Ayurveda treatises have described several herbal drugs which are used as different home remedies and are assume to be effective in COVID-19 therapeutics and immunity modulator as a preventive solution. That's why in this present study, an challenge is made to review such herbal drugs and identify its immune modulator effect against corona virus. Tulsi, Ginger, Clove, Dalchini, Turmeric, Garlic, Marich are most effective herbal drugs used as a home remedies to improve the immunity level naturally with speedy recovery in COVID-19 cases.

KEYWORDS: Covid-19, Immunity, Herbal drugs.

INTRODUCTION:
COVID-19, a global pandemic declared by WHO, is a highly infectious and severe acute respiratory disorder caused by a pathogenic virus called SARS-CoV-2 which is transmitted to humans via contact and feeding on infected animals. The COVID-19 clinical manifestations are very similar to viral pneumonia such as fever, fatigue, cough, shortness of breath, and other complications. According to reports obtained on WHO and NCDC websites as of 12th July 2020, the coronavirus breakout in Wuhan, a city in Hubei Province of China in November 2019 as spread to more than 200 countries in the world. This global pandemic has forced many nations to lock down their social activities which in turn have adverse effects on the economy[1].

Coronaviruses belong to the subfamily Coronavirinae in the family Coronaviridae of the order Nidovirales and can cause respiratory, digestive, and nervous system diseases in humans and many other animals[2].

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Which consists of four genera namely: Alpha, Beta, Gamma, and Delta coronavirus. It is currently thought that, SARS-CoV-2 has azoonotic origin and has secondarily acquired human-to-human spreading capacity. In particular, the acquisition of 1)Mutations in the receptor-binding area, 2) A polybasic furin cleavage site (RRRAR)at the junction of subdomain 1 and 2 of the spike protein and 3) A site of O-linked glycosylation in the same area, have enabled the virus to efficiently interact with high affinity(via its spike protein) with its bona fide cellular receptor (angiotensin-converting enzyme 2[ACE-2]), to become more virulent and pathogenic, while potentially evading immune responses through O-glycan epitope maskimg[3].

Morphology and genomic structure of HCoV:
Coronaviruses are spherical or pleomorphic, with diameter of 80-120nm. The virion surface is decorated with club like projections constituted by the trimeric spike (S). The viral envelop is supported by the membrane (M) proteins the most abundant structural protein. And a small transmembrane protein known as the envelop (E) protein is also present in a low amount in the envelope. The genomic RNA and phosphorylated

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nucleocapsid (N) protein form a spiral nucleocapsid, which is located within the envelope. The coronavirus genome is comprised of a single-stranded positive-strand RNA ranging from 27 Kb to 32 Kb in length. The genomic RNA is 5'-capped and 3'-polyadenylated and contains multiple open reading frames (ORFs). The invariant gene order is 5'- replicase-S-E-M-N-3', with numerous small ORFs (encoding accessory proteins) scattered among the structural genes[4].

Entry mechanism of human coronaviruses:

The life cycle of SARS-CoV-2 in host cells- begins when S protein binds to the cellular receptor ACE2. After receptor binding, the conformation change in the S protein facilitates viral envelope fusion with the cell membrane through the endosomal pathway. Then SARS-CoV-2 releases RNA into the host cell. Genome RNA is translated into viral replicase polyproteins pp1a and 1ab, which are then cleaved into small products by viral proteinases. The polymerase produces a series of subgenomic mRNAs by discontinuous transcription and finally translated into relevant viral proteins. Viral proteins and genome RNA are subsequently assembled into virions in the ER and Golgi and then transported via vesicles and released out of the cell[5].

Symptoms and effects of covid-19:

An infected COVID-19 patient can have two major states of infection, the asymptomatic state, and the symptomatic state. The symptomatic stage can develop into Acute Respiratory Disease Syndrome (ARDS) then rising infection can lead to multi- organ failure which can be fatal to the patient. An asymptomatic patient does not exhibit any symptoms of the disease due to high immunity but is still capable of infecting others, his state is extremely dangerous for the community and transmission of the virus. It is impossible to identify an asymptomatic patient without conducting an RT-PCR (Real-time polymerase chain reaction) test. Symptomatic patients exhibit varying level of severity of the disease, most patients display mild symptoms only like fever, cough, sore throat, headache, myalgia or severe symptoms like ARDS or organ failure. In the case of COVID-19, an extreme rise in inflammatory cytokines, monocytes, etc. leads to vasodilation. Which leads to the symptoms including shortness of breath, rapid breathing and bluish skin coloration[6].

Prevention:

The prevention and management are very important issues to control COVID-19. Therefore, there is a great need for the collective efforts of the public and the government. The regular and the proper care of the homes and hospitals are very important to control this calamity. The hand cleaning with soap and sanitizer, mouth and nose coverage with mask during sneezing and coughing are essential. Touching specific parts of the face like eyes, nose, and mouth without washing the hands should be avoided as these are entry points for the virus. Avoiding person-to- person contact. Regular cleaning of the surface by the disinfectants may control the virus outbreak. It is always better to avoid the interactions with anyone; suspecting respiratory problems symptoms like sneezing, coughing, breathing problem, etc. Screening has a vital role as a preventative measure to detect a potential health problem in an individual who doesn't have any signs and symptoms. Screening should be done in a multiphase level to aid further management of the disease[7].

Immunity:

The immune system refers to a collection of cells and proteins that function to protect the skin, respiratory passages, intestinal tract and other areas from foreign antigens, such as microbes (organisms such as bacteria, fungi, and parasites), viruses, cancer cells, and toxins. The immune system can be simplistically viewed as having two "lines of defense": innate immunity and adaptive immunity. Innate immunity represents the first line or defense to an intruding pathogen. It is an antigen-independent (non-specific) defense mechanism that is used by the host immediately or within hours of encountering an antigen. The innate immunity response has no immunologic memory and, therefore, it is unable to recognize or "memorize" the same pathogen should the body be exposed to it in the future. Adaptive immunity, on the other hand, is antigen- dependent and antigen-specific and, therefore, involves a lag time between exposure to the antigen and maximal response. The hallmark of adaptive immunity is the capacity for memory which enables the host to mount a more rapid and efficient immune response upon subsequent exposure to the antigen. Innate and adaptive immunity are not mutually exclusive mechanisms of host defense, but rather are complementary, with defects in either system resulting in host vulnerability[8].

Immunomodulators:

An immunomodulator may be defined as a substance, biological or synthetic, which can stimulate, suppress or modulate any of the components of the immune system including both innate and adaptive arms of the immune response. modulation of the immune system denotes to There are generally of two types immunomodulators based on their effects: immune suppressants and immune stimulators. Specific immunomodulators administered together with antigens to boost the immune response to the vaccine constituents. For instance, a planet origin saponin used in veterinary medicine. Whereas, non-specific immunostimulators offer a generalized state of resistance to pathogens or tumors. The concept of immunomodulation relates to nonspecific activation of the function and efficiency of macrophages, granulocytes, complement, natural killer cells and

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lymphocytes and also to the production of various effectors molecules generated by activated cells. It is expected that theses nonspecific effects give protection against different pathogens including bacteria, viruses, fungi etc. and constitute an alternative to conventional chemotherapy[9].

Our body temperature and wealth of nutrients provide an ideal home for these micro-organisms to thrive. The human immune system comprises innate and acquired immunity. Natural killer (NK) cells, complement system, macrophages, antigen presenting cells (APCs) and neutrophils make up the innate immune system and mounts an immediate non-specific response to foreign microbial agents. If microbes by-pass this primary defence, the acquired immune response, comprising humoral and cell mediated components, will then act to contain the invaders. The type of antigen (fungi, virus, bacteria, toxin) processed and presented by APCs to the CD4+ T cell determines the type of cytokines secreted, which in turn, determine the differentiation of helper T (TH) cells into TH1 or TH2 cells and B-cells to give immunoglobulin sub- types. TH1 response involves the activation of macrophages, which contain and destroy mycobacteria and fungal fungal pathogens. TH1 pathway also activates cell-mediated immunity. TH2 cells, on the other hand, effect immunoglobulin differentiation and antibody secretion, and therefore mediate humoral immunity. CD8 cytotoxic T cells induce apoptosis in antigen-laden cells[9].

Ayurveda purview:

Ayurveda is a comprehensive scientific medicinal system indigenous to India. The term Ayurveda means 'knowledge of life'. Which comprises two Sanskrit words, Ayu (life) and Veda (knowledge or science). Four Vedas, considered as a the oldest Indian literature (5000-1000 BC) contain information about natural remedies. Ayurveda was established as fully grown medicinal system. Charaka Samhita (focussing on internal medicine) and Susruta Samhita (focussing on surgery) were written systematically and considered as classical text of Ayurveda. Vital details of Charaka Samhita and Susruta samhita were compiled together and updated additionally in Astanga Sangraha and Astanga Hrdaya. Some other ancient classics which include minor work of Ayurveda includes Madhava Nidana (focusing on diagnosis of disease), Bhava Prakasa (focussing on additional information related to plant and diet), arngadhara Samhita (focusing on formulation and dosage form). Ayurveda was divided into eight major clinical subdivisions-Kayacikitsa (internal medicine), Salya Tantra (surgery), salakya (diseases of supra- clavicular origin), Kaumarabhrtya (paediatrics, obstetrics and gynaecology), Bhutavidya (psychiatry), Agada Tantra (toxicology), Rasayana Tantra (rejuvenation and geriatrics), Vajikarana (aphrodisiology and eugenics)[10].

Concepts underpinning ayurvedic medicine:

The 3 basic principles, called doshas (vata, pitta, and kapha), are derived from 5 elements of Indian philosophy. Ayurveda's doshas can be identified as regulatory control factors for fundamental physiologic processes in living systems that maintain their identity throughout biologic history: vata and its subdoshas regulating input/output processes and motion; pitta and its subdoshas regulating throughput, turnover, and hence energy; and kapha and its subdoshas regulating storage, structure, and lubrication. Factors such as food, activity, the climate and stress can, however, disrupt or destroy these functions. Ayurveda seeks to normalize body functions with varied techniques including advice onfood and activity, internal herbal preparations, purification treatments (panchakarma), and surgical methods (shalya chikitsa). Oral administration routes play a major role in influencing individuals' doshas, via the ingestion of food, spices, and medicinal plants. These elements are influencing doshas in different ways: stabilizing, disturbing, and supporting the body's healthy state[11].

Role of herbal drugs as immunity booster:

Plants are always the key source of drug or treatment strategy in different traditional medicinal systems. In recent years, many people are choosing to plant based medicines or products to improve their health conditions or as curative substance either alone or in combinations with others. According to the WHO, herbs or herbal products are used by the large number of populations for basic healthcare needs. Herbal medicine includes herbs, herbal materials (like plant parts) or preparations, processed and finished herbal products, active ingredients[10].

1. Tulsi:

Many in-vitro, animal and human experimental scientific studies showed that; due to presence of eugenol, phenolic compounds, linoleic acid, etc. compounds Tulsi has antimicrobial (including antibacterial, antiviral, antimalarial), anti-diarrheal, anti-oxidant, anti-inflammatory, hepatoprotective, cardioprotective, reno-protective, analgesic, antipyretic, immunemodulatory properties and is thus recommended as a treatment for a range of diseases including features like cough, fever, asthma, anxiety, diarrhea, gastric, cardiac and genitourinary disorders. Due to its anti-inflammatory and antioxidant properties, it protects against toxic chemical-induced injury, enhance the antioxidant enzymes and protect cellular organelles and membranes by clearing damaged free radicals. The compounds such as ursolic acid, carnosol, rosmarinic acid, cirsilineol, apigenin, eugenol, and cirsimaritin present in O. sanctum increase haemoglobin concentration, enhance SRBC agglutinin titers, decrease cyclo-oxygenase (CoX)-2 and lipoxygenase (LOX)-5 enzymes activity, suppress NF-

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kB classical pathway, up regulation of IL-2, IFN-g and TNF-a, down regulation of IL-1b and produce of SRBC antigen-specific antibodies, which represent a major defense mechanism to assess T-cell-dependent antibody responses i.e. Tulsi by enhancing immune response boost the defense mechanism against the infection. Several studies have shown that Tulsi (aqueous and methanol extract of leaf and seed oil) besides improving vital capacity also is an immune-modulator and regulator as it enhances immune response by increasing T-helper and NK cells; phagocytic activity and index with the rise in lymphocyte count, neutrophil count and antibody titer [12].

2. Ginger:

Ginger is the rhizome of Zingiber officinale Roscoe in the family Zingiberaceae and has been used as a food, spice, supplement and flavoring agent and in traditional medicines for more than 3000 years in countries. Ginger has been used in traditional medicines to treat diseases and symptoms, such as colds, headache, nausea, upset stomach, diarrhea, arthritis and rheumatism, or used as a carminative, antiflatulent and digestant. Furthermore, ginger is known to have pharmacological activity against natural, chemical and radiation-induced toxicities, such as radioprotective, hepatoprotective, nephroprotective, neuroprotective, gastroprotective and reproductive-system-protective effect. The bioactive compounds of ginger such as nevirapine, b-sitosterol, 6 gingediol, germacrene, methyl-6-shogaol, 6-gingerol, a-linalool, 6-shogaol, gingerdion, zingiberene, etc., are known to inhibit viral replication; among these the most potent inhibitors of reverse transcriptase (RT) enzyme is b-sitosterol, which is predicted to be used as non nucleoside reverse transcriptase (NNRTIs) HIV-1 inhibitors. It is reported that Ginger contains TNF-a which is also known as an anti-influenza cytokine.The rhizome of Ginger and its main components like gingerols, shogaols, etc inhibit prostaglandin and leukotriene biosynthesis, inhibit cyclooxygenase and lipoxygenase activities, inhibits the synthesis of pro-inflammatory cytokines such as IL-1, TNF-a, and IL-8 without any significant effect in IL-6 levels; inhibit the excessive production of NO, PGE (2), TNF-a, and IL-1beta, reduce the elevated expression of NFkB and TNF-a, downregulate inflammatory iNOS and COX-2 gene expression, inhibit thromboxane synthetase, raise levels of prostacyclin without a concomitant rise in PGE 2 or PGE 2 alpha, inhibit platelet aggregation, decrease age-related oxidative stress markers and enhance

Fibrinolysis. The concentration of IgM and eosinophil count in non-smokers was significantly increased in a comparative study of the effect of ginger extract among male smokers and non-smokers, whereas the concentration of hemoglobin and lymphocyte count in smokers was strongly increased. This indicates that in non-smokers, ginger results in a stronger antibody response or humoral immunity than in smokers[12].

3. Clove:

Cloves are an aromatic herb that has many useful purposes. Approximately, 72-90% of the essential oil extracted from cloves has Eugenol. Other are Acetyl eugenol, Beta-caryophyllene and vanillin, Crategolic acid, tannins, gallotannic acid, methyl salicylate (painkiller), Flavonoids eugenin, kaempferol, Triterpenoids like oleanolic acid. The dried buds of cloves contain antiseptic, and anti-fungal agent. It also holds aphrodisiac and circulation-stimulating capacities. The oil of cloves has been used in a variety of health conditions including indigestion, generalized stress, parasitic infestations, cough, toothaches, headache, and blood impurities. Clove is used extensively in dental care for relieving toothache, sore gums and oral ulcers. Gargling with clove oil can also aid in sore throat conditions and bad breathe. Clove oil clears the respiratory passages, acting as an expectorant for treating many upper-respiratory conditions including colds, bronchitis, sinus conditions, cough and asthma. Not only purifies the blood, but also aids in stabilizing blood sugar levels, and may have benefits for diabetic individuals. Clove's antiviral and cleansing properties purify the body, augmenting our resistance to disease[13-16].

4. Dalchini:

The Cinnamon popularly known as Dalchini (Cinnamomum zeylanicum), belongs to the family Lauracae. Cinnamon has also been used for its medicinal properties for thousands of years. Made from the inner bark of the cinnamomum tree, its use has been dated as far back as ancient Egypt. Cinnamon is an immune simulator, protecting the body from bacterial or viral attacks. It helps your body fight infections and repair tissue damage. All the antioxidants are super powerful when it comes to bringing those anti-inflammatory properties. Cinnamon also gives us manganese, calcium, fiber and iron. Cinnamon also fights inflammation and helps ward off infections and herbal damaged tissue. Containing large amounts of polyphenol, cinnamon outranked “superfoods” like garlic and oregano in a study comparing the antioxidant, antitumor, antihypertensive, antilipemic, antidiabetic, gastroprotective, and immunomodulatory effects[17]. In addition to being lipid lowering and cardiovascular-disease- lowering compound, cinnamon has also reported to have activities against neurological disorders, such as Parkinson’s and Alzheimer’s diseases. Cinnamon is a coagulant and prevents bleeding. Cinnamon also increases the blood circulation in the uterus and advance tissue regeneration[18].

In one study, cinnamon at high dose (100mg/kg) showed immune-stimulant activity as it significantly increased

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the phagocytic index, serum immunoglobulin levels and antibody titer and decreased the percentage reductions in neutrophil count. Cinnamon low dose (10mg/kg) increased serum immunoglobulin levels only. This showed that high dose increases both cell mediated and humoral immunity whereas low dose showed effect only on humoral immunity. The studies also suggest that cinnamaldehyde can act as a strong regulator of monocyte/macrophagemediated immune responses by inhibition of PI3K, PDK1 and NF-kB activation of signaling components. In addition to this, by the activation of CD29 and CD43, it blocked cell migration cellecell adhesion induced but not cell-fibronectin adhesion and it was able to suppress both the production of nitric oxide (NO) and up regulation of surface levels of co-stimulatory molecules (CD69 and CD80) and pattern recognition receptors (TLR2 and CR3).

5. Turmeric:

Turmeric (Curcuma longa), also known as “Indian saffron” due to its brilliant yellow colour, is a spice herb, member of the ginger family (Zingiberaceae) native to the Indian subcontinent and Southeast Asia, having more than a two centuries old scientific history. Turmeric obtained from ground-dried root contains different percentages of volatile and non-volatile oils, proteins, fats, minerals, carbohydrates, curcuminoids and moisture. Commercially available curcumin is a combination of three molecules, together called curcuminoids. Curcumin is the most represented (60–70%), followed by demethoxycurcumin (20–27%) and bisdemethoxycurcumin (10–15%). Besides curcuminoids, the other active components of turmeric include sesquiterpenes, diterpenes, triterpenoids[19].

Turmeric has various useful properties with antioxidant activities. Turmeric has anti-inflammatory, anticancer, anti-diabetic, hypolipidemic, antimicrobial, anti- fertility, anti-venom, hepatoprotective, nephroprotective, anticoagulant property. The plant has also shown to possess anti HIV activity to combat AIDS[17]. The immunomodulatory abilities of curcumin arise from its interaction with various immunomodulators, including not only cellular components, such as dendritic cells, macrophages and both B and T lymphocytes, but also molecular components involved in the inflammatory processes, such as cytokines and various transcription factors with their downstream signalling pathways. Curcumin supplementation in rabbit diet (2,4and 6g/kg) significantly increased serum levels of IgG and IgM, thus suggesting that curcumin can also improve immune response[20].

6. Garlic:

Garlic (Allium sativum) is bulbous perennial plant with a powerful onion such as aroma and pungent taste that has been used as flavoring agent, condiment, and for medicinal purposes for over 5,000 years. Garlic contains a variety of bioactive constituents includindg sulfur compounds such as alliin, allicin, ajoene, allylpropyl disulfide, diallyl disulfide (DADS), diallyltrisulfide (DATS), S-allylcysteine (SAS); peroxidases and alliniase like enzyme, amino acids and important trace elements like Se, Ge and Te. Garlic is frequently used to treat aches and pains, leprosy, diarrhea, infections, dandruff, respiratory disorders. Garlic has been employed for management of blood pressure, atherosclerosis, high cholesterol, heart attack and coronary heart disease. Aged garlic has more potent immunomodulatory effects than raw garlic. Garlic is an effective therapeutic candidate to prevent the recurrent aphthous ulcer. Conditions like gout, rheumatoid arthritis, osteoarthritis, diabetes, allergic rhinitis, traveler’s diarrhea, bacterial and fungal infections, cold and flu are also known to cured by garlic. Other uses of garlic include treatment of fever, whooping cough, headache, stomach ache, sinus congestion, psoriasis, hair loss and hemorrhoids[21].

7. Marich:

It has been also found to increase bioavailability, thus enhance the therapeutic efficacy of many drugs, vaccines and nutrients and have immune-modulatory, anti-oxidant, antiplatelets, antihypertensive, anti-asthmatic, antipyretic, analgesic, anti-carcinogenic, anti-inflammatory, anti-diarrheal, antispasmodic, anxiolytic, antidepressants, hepatoprotective, anti-ulcer, anti thyroids, antiapoptotic, anti-metastatic, antimutagenic, antibacterial, antifungal and anti-amoebic properties. The extract and its constituents like piperine, regulate the balance of the cytokines production of Th1, Th2, Th17, and Treg cells, reduce the accumulation of inflammatory cells, inhibit the expressions of GATA3, IL-4, IL-6, IL-1b, RORgt, IL-17A and TNF-a, increase INF-g and IL-10 secretions in BALF (Broncho-alveolar lavage fluid) and increase macrophage activation and T and B cell proliferation. Beside this, Marich possess cytotoxic activity, suppresses the levels of total IgE, anti-OVA IgE, anti-OVA IgG1 and histamine release in serum, ameliorates fibrosis and infiltration of inflammatory cells, inhibits the allergic responses, inhibitsTh2/Th17 responses and mast cells activation, inhibits NF-kB, c-Fos, cAMP response element-binding (CREB) and activated transcription factor (ATF-2); suppresses PMA-induced MMP-9 expression, inhibits PKCa/extracellular signal regulated kinase (ERK) 1/2 and reduces NF-kB/AP-1 activation. In addition, piperine also inhibits the Pglycoprotein (P-gp) and CYP3A4 functions. Piper nigrum is found to have dose dependent antifertility effects on mice[22].

CONCLUSION:

COVID-19 viral spectre outbreak is spreading across different countries at an increasingly alarming rate.

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Currently, yet no any vaccine or medicine could be developed to cure COVID-19 and Scientist also utilizing hydroxychloroquine to treat COVID-19 but could not get positive response and has side effect. Immune systems in body play an important role to fight against unhealthy environment and microbes such as virus, bacteria, fungus etc. In the current pandemic infection of COVID-19 it is clear that those with weak immune system are highly susceptible to this infection and worst outcomes. In the case of infectious pandemics like this "prevention is always better than cure". In this regard immune enhancing herbs may definitely be helpful for the body to fight COVID-19 infection. Tulsi, Ginger, Clove, Dalchini, Turmeric, Garlic, Marich these botanical plants having low cost, minimum toxicity and almost found everywhere in country, it has potential to enhance immunity to fight against COVID-19 and other infectious disease and play an important role to becomes fit and healthy India and world.

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FORMULATION AND EVALUATION OF ANTIFUNGAL MICROEMULSION BASED GEL FOR TOPICAL DRUG DELIVERY USING MILLETIA PINNATA

Bhagyashri Gadave[1]*, Atish Velhal[2] and Dr. Vivekkumar Redasani[3]

[1]Department of Pharmaceutics, YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara 415011, Maharashtra, India.

[2]Assistant Professor, YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara 415011, Maharashtra, India.

[3]Principal, YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara 415011, Maharashtra, India.

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***Corresponding Author**
Bhagyashri Gadave
Department of Pharmaceutics, YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara 415011, Maharashtra, India.

ABSTRACT

The goal of this study was to develop and test a topical gel containing an Itraconazole microemulsion (ITZ). A preformulation research was conducted before the formulation of Itraconazole microemulsion. To determine the maximal solubility of ITZ in oils, surfactants and co-surfactants were tested to determine excipient potential. In order to microemulsion region, with Karanj oil as the oil phase, Tween 80 as the surfactant, and Isopropyl alcohol (IPA) as the co-surfactant, a pseudoternary phase diagram was created. The optimized ME of ITZ was characterized by its qualitative & Quantitative test & incorporated into polymeric gels of Carbopol (CBP), Xanthan gum, Carbopol 934, Carboxymethyl cellulose (CMC), Carboxymethyl -Tamrind gum (CMTG). ME evaluated by % transmittance, Viscosity, pH, particle size, zeta potential, Physical appearance, Drug content, pH, spreadability, viscosity, In -vitro release. Stable ME was obtained when Karanj oil was taken as oil phase, Tween 80 as surfactant & IPA as co-surfactant at the weight ratio of 5:45:50. The optimized ME based gel shows pH range 6.0- 6.34, Spreadability in the range of 0.56-1.06gm.cm/sec. The viscosity study indicated pseudoplastic behavior of all ME based gel formulations. Amongst the studied ME gels CBP: CMTG containing gels showed maximum drug release at the end of 6h. The prepared MEG show better release profile than marketed preparation.

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DIRECTOR
Yashoda Technical Campus
Satara

KEYWORDS: Itraconazole, Microemulsion based gel, Pseudoternary Phase Diagram, Topical drug delivery.

INTRODUCTION

Approximately two-thirds of the world's population is infected with a common fungal illness.[1] Fungal infection is a frequent infection that affects two-thirds of the world's population. In recent years, the prevalence of fungal infections caused by fungi including Candida, Aspergillus, and Cryptococcus has increased. Skin diseases caused by fungi are known as mycoses. Candida skin infections can affect practically any part of the body, but they're most common in intertriginous areas, where two skin patches rub or touch.[2,3]

Itraconazole (ITZ) is a triazole antifungal with a wide range of activity. It's a medication from the BCS class II. Bioavailability of Itraconazole in conventional dose formulations was around 15-20%. It has a 6-hour biological half-life. Constipation, abdominal pain, headache, and, in rare cases, heart failure have all been reported as side effects of ITZ. The fact that ITZ is contraindicated in patients with renal and/or hepatic impairment is also a drawback.[4,5]

Topical treatments, such as creams and ointments, are sticky and need rubbing, which can make patients uncomfortable. As a result of their numerous advantages over other semisolid preparations, gels have gained prominence in both the pharmaceutical and cosmetic fields.[6] Gels are characterised as a semi-rigid system in which the dispersion medium's moment is limited by interlacing three-dimensional networks of particles. They are non-invasive and patient-friendly, are less greasy, and can be easily removed from the skin. They're also affordable, have a localised action with little side effects, boost medicine absorption, reduce dose frequency, and stabilise drug distribution patterns.[6,7] Despite the many benefits of gels, one important drawback is the delivery of hydrophobic medicines. As a result, a microemulsion-based approach is being employed to break through this barrier, allowing even a hydrophobic medicinal moiety to benefit from the special features of gel.[8]

Microemulsions (MEs) have gained in popularity and attention in recent years due to their unique properties. Industrial laboratories, as well as academic researchers and those working in the pharmaceutical industry, have shown an interest in these compounds, which has led to their use in a variety of administration methods. The stable MEs are simple to make and can improve the solubilizing efficacy of both hydrophilic and lipophilic pharmaceuticals, hence increasing drug permeability.[9] ME's low viscosity, on the other hand, makes it difficult to

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apply to the skin and reduces patient compliance.[10] When compared to solution, gel, or formulations, MEs or ME gels dramatically improve medication absorption. Natural polymers are cost-effective in distribution systems because they are readily available. They're also biodegradable, biocompatible, and easily accepted by regulatory bodies.[11]

Polymers including carbopol (CBP), hydroxypropyl methylcellulose (HPMC), carboxymethyl-tamrind gum (CMTG), carboxymethyl cellulose (CMC), and in the creation of ME gels, natural polymers such as xanthan gum (XG) have been characterized.[29,12-14]

Karanj oil, a non-edible semi-drying fixed oil derived from seeds of Pongamia pinnnata belonging to the Fabaceae family, is one of the natural.[9] According to the literature, Karanj oil is a therapeutic oil that is mostly used to treat itches, abscesses, and skin problems.[10] As a result, Karanj oil can be utilised as an oil phase in the formulation of microemulsion-gels for topical delivery of drugs that are weakly water soluble, potentially improving absorption and prolonging drug release.

As a result, it was proposed to develop and test ME including topical gels of CBP, XG, TG, CMTG, and CMC for better hydrophobic drug delivery. Further research was carried out to determine the viscosity and drug release of the produced gels. ITZ's gastrointestinal adverse effects may be mitigated by a recently developed ME-based gel.

2. MATERIALS AND METHODS

2.1 Materials

Aurochem Pharmaceuticals Pvt. Ltd., Palghar, provided ITZ. Loba chemie, Mumbai, provided Tween 80, isopropyl alcohol (IPA), olive oil, Tween 20, polyethylene glycol 400 (PEG400), and carboxymethyl cellulose (CMC). S.D Lab chemical centre in Mumbai provided xanthan gum and oleic acid. All additional chemicals were acquired from Loba Chemie in Mumbai and were of analytical quality.

Table 1. Formulation of microemulsion based gels.

Formulation code	F1	F2	F3	F4	F5	F6	F7	F8	F9
Gel									
Carbopol-934 (gm)	0.5	1.0	1.5	-	-	-	-	-	-
Xanthan gum (gm)	-	-	-	0.5	1.0	1.5	-	-	-
CBP:XG (1:1) (gm)	-	-	-	-	-	-	1.0	-	-
CBP:CMC (1:1) (gm)	-	-	-	-	-	-	-	1.0	-
CBP:CMTG (1:1) (gm)	-	-	-	-	-	-	-	-	1.0

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Water (ml)	100	100	100	100	100	100	100	100	100
Microemulsion									
Itraconazole (gm)	2	2	2	2	2	2	2	2	2
Karanj oil (ml)	5.41	5.41	5.41	5.41	5.41	5.41	5.41	5.41	5.41
Tween-80:IPA (6:4) (ml)	45	45	45	45	45	45	45	45	45
Water (ml)	50	50	50	50	50	50	50	50	50
Methyl paraben (gm)	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Propyl paraben (gm)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

2.2 Solubility study of ITZ

The oils and excipients were chosen due to itraconazole high solubility in them. Based on the literature analysis, Karanj oil was chosen as an efficient excipient for micro- emulsion formation.

The solubility of itraconazole in several oils (Karanj oil, Olive oil, Oleic acid) was studied to determine the best oil for usage as the oil phase in microemulsion. Itraconazole solubility in several surfactants (Tween-20 and Tween-80) and cosurfactants (Isopropyl alcohol, propylene glycol PEG-200, PEG-400) was also investigated. In stoppered vials (capacity 10mL), an excess amount of itraconazole was added to 3mL of the specified oil, surfactant, and cosurfactant, and then preliminary mixing was carried out over magnetic stirrer for a few minutes. These vials were then held at 37±0.5^{0}C for 72 hours in a mechanical bath shaker. After that, the equilibrated samples were centrifuged (Remi) for 15 minutes at 3000 rpm. The supernatant was collected, membrane was filtered and spectrometric sample measurements at 262nm. determined solubility after proper dilution by methanol.Each experiment was carried out three times.[15]

2.3 Construction of pseudoternary phase diagram

The difference fraction of mixed surfactant was often used in the building of a phase diagram, and the surfactant and cosurfactant optimal ratio (Km) was estimated using the microemulsion area. Km was investigated using a simple pseudoternary phase diagram. The generation of microemulsions utilising a four-component system consisting of an oil phase, a non-ionic surfactant, a cosurfactant, and purified water was investigated using pseudoternary phase diagrams (aqueous phase).

Titration of homogeneous liquid mixes of water, surfactant, and cosurfactant with oil phase at ambient temperature yielded the pseudo ternary phase diagram. Surfactant and co-surfactant were combined in a 1:9 to 9:1 ratio. The nine samples were mixed consistently and

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independently with water, and then the oil was added drop by drop to the mixture. Water content was set at 2.0 gm, and the total amount of surfactant and co-surfactant was also set at 2.0 gm. To allow for equilibration, samples were agitated by a vortex shaker during the titration. The combination was visually evaluated for transparency after the addition of an aliquot of oil, until the system became slightly hazy. The microemulsion window was discovered to exist as the area where clear and transparent formulations may be seen upon visual inspection. The water ratio was held constant, and the oil, surfactant, and cosurfactant formed the pseudoternary phase diagram.[16]

2.3 Construction of Ternary Phase Diagram

The best surfactant and cosurfactant weight ratio (Km) was chosen. The contents of mixed surfactant and oil in the mixtures varied from 9:1 to 1:9. A homogeneous oil surfactant–cosurfactant blend was created, where Km was fixed and the contents of mixed surfactant and oil in the mixtures varied from 9:1 to 1:9. The total amount was kept at 1.0 g. Drop by drop, purified water was added to each mixture. To allow for equilibration, samples were agitated with a magnetic stirrer during the titration. The combination was visually evaluated for clarity after an aliquot of water was added until the system became slightly cloudy.[17]

2.4 Preparation of ITZ ME

The Smix ratio with the largest microemulsion region was chosen. Oil and Smix were blended in various quantities. Itraconazole was dissolved in a mixture of oil and Smix at room temperature using magnetic stirring. Dropwise additions of double distilled water to the oily mixture were made until a clear and transparent microemulsion was formed. With mild magnetic stirring, the mixture was allowed to stabilise and reach equilibrium for 15–20 minutes. Itraconazole-containing microemulsions were then kept at room temperature.[18]

2.5 Qualitative and Quantitative tests for ME

Dilution test

The dilution test was performed by diluting 1 ml of prepared ME(s) to 100 ml and observed for clarity/turbidity/phase separation. It is confirmatory test of microemulsion to know which type of microemulsion was formed.

Centrifugation

Centrifugation test was used to evaluate physical stability of microemulsions. Microemulsions were centrifuge (Remi Laboratories, Mumbai, India) at 5000 rpm for 10 min and system was evaluated for creaming or phase separation by visual observation.[19]

pH of microemulsion

pH of microemulsion was determined by using digital pH meter (Systronics).

Transmittance (%T)

The percentage transmittance of 2ML ME(s) was checked against distilled water using UV-VIS spectrophotometer at 650 nm.

Drug Content Studies

In a 50 ml volumetric flask containing methanol, a microemulsion equivalent to 5 mg of itraconazole was placed and swirled for 30 minutes. Methanol was used to increase the volume to 50 mL. The resulting solution was further diluted by 2 ml of methanol using a membrane filter of 0.45µm.The absorbance of the solution was measured spectrophotometrically (Shimadzu UV, Japan) at 262nm.[20]

Dispersion stability studies

For 30 minutes, the formulations were centrifuged at 3500 rpm. For the heating and cooling cycle, no phase separation formulations were used (freeze thaw cycle). Six cycles were performed in a hot air oven at temperatures ranging from 4°C (refrigerator) to 45°C, with storage at each temperature for at least 48 hours. For further research, the formulations that were stable at these temperatures were chosen.[15]

Transmission electron microscopy

Transmission electron microscopy was used to examine the morphology of itraconazole microemulsion (CM200, Philips, FEI Company). One drop of diluted samples was put on film-coated copper grids, dried, and studied under the electron microscope after being negatively stained with 2 percent phosphotungstic acid (PTA).[21]

Globule size and zeta potential measurements

The globule size and zeta potential were assessed using the zetasizer nano-zs (Malvern instrument). At a temperature of 25°C, the experiment was carried out. A 1ml sample was diluted with double distilled water.[22] The globule size and zeta potential were assessed using

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the zetasizer nano-zs (Malvern instrument). At a temperature of 25°C, the experiment was carried out. Double distilled water was used to dilute a sample of 1ml.[22]

2.7 Preparation of ME based gels of ITZ

Distilled water was used to make blank gels of various polymers. In a nutshell, the polymer was dispersed in 100 mL distilled water and blended for 60 minutes using a mechanical mixer (Remi). For carbopol gels, triethanolamine was utilised as an alkalising agent.[23] For the ME preparation, the preservative was first thoroughly combined with a mixture of oil and Smix. The medicine, Itraconazole, was then dissolved in the aforesaid mixture at room temperature using magnetic stirring. Dropwise additions of double distilled water to the oily mixture were made until a clear and transparent microemulsion was formed. With mild magnetic stirring, the mixture was allowed to stabilise and reach equilibrium for 15–20 minutes. All itraconazole-containing microemulsions were then kept at room temperature.[18] The gels and microemulsions were combined in a 1:1 ratio.[24] The following table lists the formulation batches in detail. 1.

2.8 Characterization of ITZ containing ME based gels

Attenuated total reflectance – Fourier transform infrared spectroscopy

The infraround spectrophotometer of ITZ, ME, was utilised in order to get a reduced total reflectance-Fourier transform infraroad (ATR-FTIR) (Shimadzu, IR Affinity, Japan).The samples were delivered to the ATR compartment for analysis. At an average of 25 scans and a resolution of 4/cm, the spectra for the range 600-4000/cm were acquired.

Physical examination

Prepared ME based gel formulations were investigated for physical characteristics like colour, homogeneity and phase separation.[25]

Drug Content

Drug content of emulgel was measured by UV spectrophotometer. 1 gm of emulgel was diluted to 50 ml with methanol. 2ml of this solution was further diluted methanol. The absorbance of the solution was measured spectrophotometrically (Shimadzu UV, Japan) at 262nm.[26]

Spreadability study

1gm of itraconazole emulgel was placed in a 1 cm diameter circle pre-marked on a glass plate, which was then covered with a second glass plate to assess spreadability. The upper glass plate was permitted to rest for 5 minutes with a weight of 500 grams on it. The gel spreading was noted from the change in diameter of gel placed.[27]

Determination of pH

The pH of itraconazole emulgel was determined by using digital pH meter (Systronics), at ambient room temperature.[28] The calibration of pH meter was done with buffered solution before each use.

Rheological Studies

The viscosity of the different emulgel formulations was determined at 25°C using a cone and plate viscometer (Brookfield rheometer RS plus).[29]

In vitro drug release studies

A Franz diffusion (FD) cell was used in the in vitro drug release research (with effective diffusion area 3.14 cm2 and 25 ml cell volume). The formulation was applied to the FD cell's egg membrane, which was sandwiched between the donor and receptor compartments. As a dissolving medium, phosphate buffer pH 7.4 was utilised. A circulating water jacket kept the temperature of the cell at 37 0C. The solution was continuously stirred using a magnetic bead while the entire assembly was kept on a magnetic stirrer. As a control, a similar blank set was run at the same time. At appropriate time intervals, a sample (1 ml) was taken and replaced with equal volumes of fresh dissolving media. After proper dilutions, samples were tested for drug content using a UV visible spectrophotometer (Shimadzu UV1800). The total percentage of drug released was computed.[30]

3. RESULTS AND DISCUSSION

3.1 Solubility of ITZ

ITZ's physicochemical features indicate that it could be useful for topical medication delivery. Karanj oil (108.40±1.59) had the highest ITZ solubility among the selected oils that were examined, hence it was chosen as an oil. Tween 80 (246.62±16.08) demonstrated reasonable solubilizing capability for ITZ among the surfactants . ITZ is most soluble in the co-surfactant isopropyl alcohol (IPA) (Freely soluble).

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Table 2: Solubility of itraconazole in various oils, surfactants and co-surfactants.

	Vehicle	solubility of itraconazole (mg/ml)
Oils	oleic acid	64.02±1.32
	Karanj oil	108.40±1.59
	Olive oil	25.68±1.37
Surfactants	Tween-20	190.12±17.12
	Tween-80	246.62±16.08
Co-surfactants	Isopropyl alcohol	Freely soluble
	Propylene glycol	151.89±18.3
	PEG-200	110.58±15.52
	PEG-400	125.65±16.3

3.2 Construction of Pseudoternary Phase diagram

The pseudoternary phase diagram of oil (Karanj oil)/IPA / Tween 80/ water system were constructed as shown in Figure. The region giving clear and transparent formulation was considered as the ME window and was marked in pseudoternary phase diagram. The best weight ratio of surfactant and cosurfactant (Km) was discovered to be 6:4, thus for subsequent investigation, the best surfactant combination (Smix) comprising Tween 80 and IPA in a 6:4 ratio was blended with the highest oil (Karanj oil).

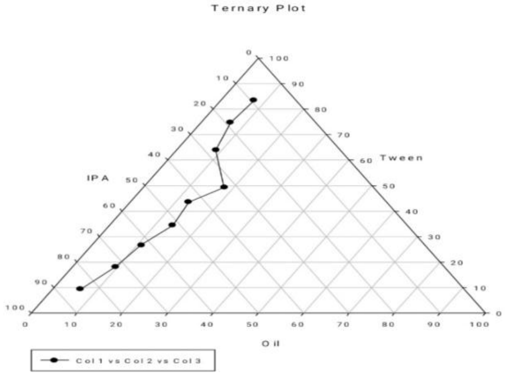

Figure 1: Pseudoternary phase diagram of the system containing Karanj oil, Tween 80, IPA and water.

3.3 Ternary Phase Diagram

The region of ME and concentration ranges of components used for formulation of ME were determined by phase studies. The effect of different surfactant /cosurfactant weight ratios on extent of stable ME region was also studied. The phase diagram of the system including oil, Smix, and water was created and is shown in fig. The microemulsion zone (ME region) in the figure is black, whereas the non-ME region is white. It is evident from the figure that tween80 and IPA could give considerable micro emulsification region (>40%).[15]

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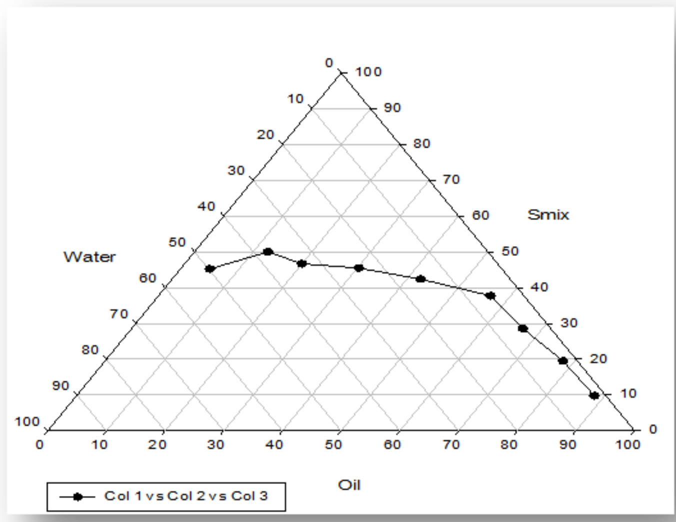

Figure 2: Phase diagram of the system containing Karanj oil, mixed surfactant and water.

3.4 Preparation of ITZ MEs

The Smix ratio with the highest ME region was chosen from the ternary phase diagram. When the weight ratios of Oil: Smix : water of 5:45:50 [M1], 10:45:45 [M2], and 10:50:45 [M3] were utilised, oil-in-water ME was generated.

3.5 Qualitative and quantitative tests of MEs

Results of qualitative and quantitave tests of all prepared MEs are given Tables.

Dilution Test

Except for formulation M1, all microemulsions generated showed phase separation and turbidity.

Centrifugation

Centrifugation test was performed to evaluate physical stability of micro-emulsions. Formulations M2 and M2 showed creaming /phase separation while other formulation was stable at centrifugation.

pH of microemulsion

The pH values of microemulsions were varied from the range 5.06 to 5.15 which was acceptable pH of skin.[31] This is an important parameter as the skin pH ranges between pH 5.0-6.5.

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Transmittance (%T)

Transmittance for all formulations are given in table and found to be in the range of 71.2 to 98.3 %. Formulation M3 shows less transmittance due to turbidity while formulation M1 shows high transmittance due to clarity.

Drug Content Studies

Dispersion stability studies

The formulations M1 stable at these temperatures were selected for further studies.

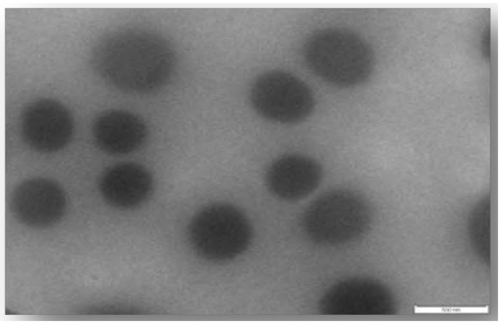
From above results the formulation M1 shows more stability than other formulations. So, M1 microemulsion was further incorporated into gelled base.

Table 3: Dilution, Centrifugation, pH, Transmittance, Drug content, Dispersion stability studies results.

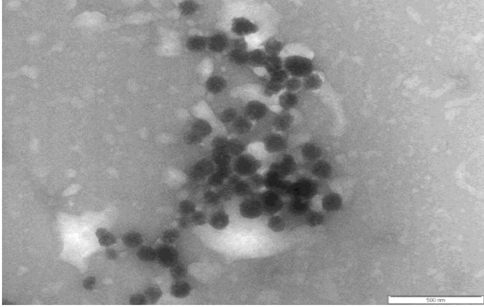
Formulation code	M1	M2	M3
Dilution test	No phase separation	Phase separation	Phase separation
Centrifugation/ creaming	No	Yes	Yes
pH	5.15	5.11	5.06
Transmittance	98.3	75.5	71.1
Drug content	99.3	98.5	95.1
Dispersion stability	Stable	Unstable	Unstable

Transmission electron microscopy

In the transmission electron microscope, the globules of optimised ME seemed to be virtually spherical in shape. In the light environment, the globule appeared dark (Fig.).The average droplet size of optimized ME was 136.4 nm. The globule size of optimized ME increases as compared optimized blank ME.



Test ME TEM



Blank ME TEM

Fig 3: Transmission electron microscopy.

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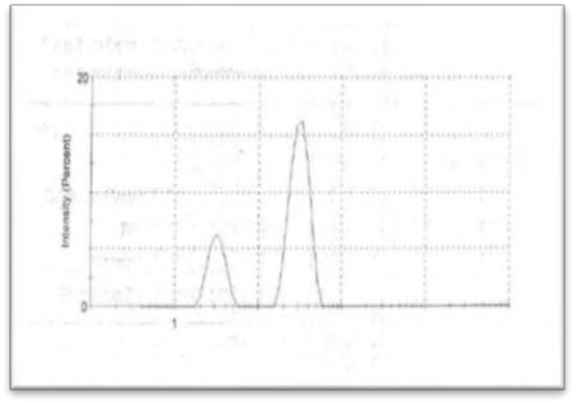
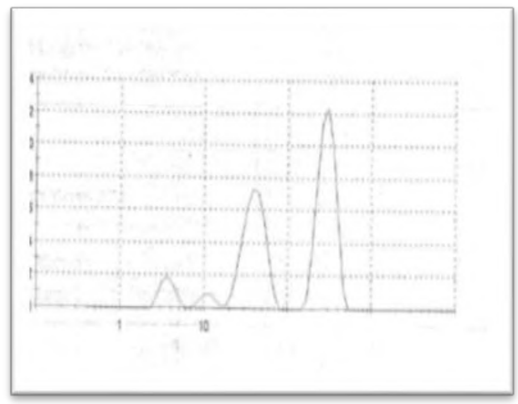
Measurement of globule size and zeta potential

Globule sizes of microemulsion were found to be 885.5dnm and 136.4dnm respectively test and blank ME formulations. The small globule size of microemulsion was due to large percent of Smix. Similarly, zeta potentials were observed to be -0.118mv and 0.00365mv respectively test and blank ME formulations.

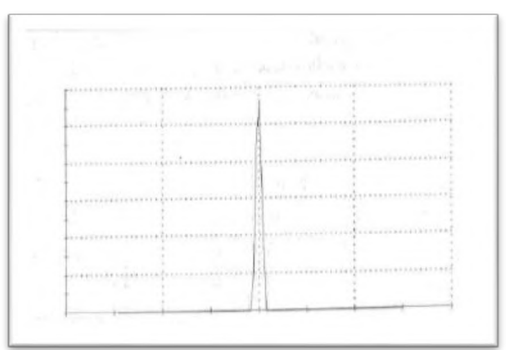


Itraconazole unloaded size distribution

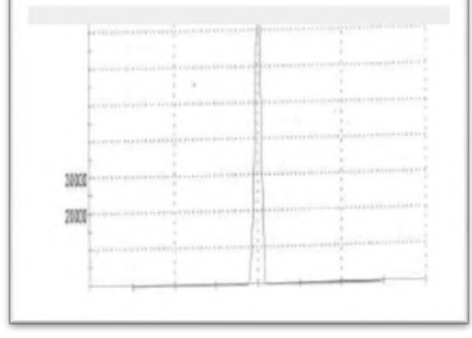


Itraconazole loaded size distribution

Figure 4: Globule size distribution.



Itraconazole unloaded zeta potential



Itraconazole loaded zeta potential

Figure 5: Zeta potential.

Table 4: Zeta potential and Globule size distribution.

Zeta potential		Globule size distribution	
Itraconazole unloaded ME (blank)	Itraconazole loaded ME (test)	Itraconazole unloaded ME (blank)	Itraconazole loaded ME (test)
0.00365	-0.118	136.4dnm	885.5dnm

Evaluation of ME gel

Melting Point

The melting point of itraconazole was found to be 166.2^{0}C. The reported melting point of drug was 166-170^{0}C.

FTIR Spectrum of Interoretation

Itraconazole's FTIR spectra revealed peaks at 1583.27 (C-N stretching), 1700.91 (C=O stretching), 1187.94 (C-H aromatic), 1141.65 (C-N stretching), 3440.39 (aromatic C-H stretching), 2927.41, and 2856.66 (C-N stretching) (aliphatic C-H stretching).

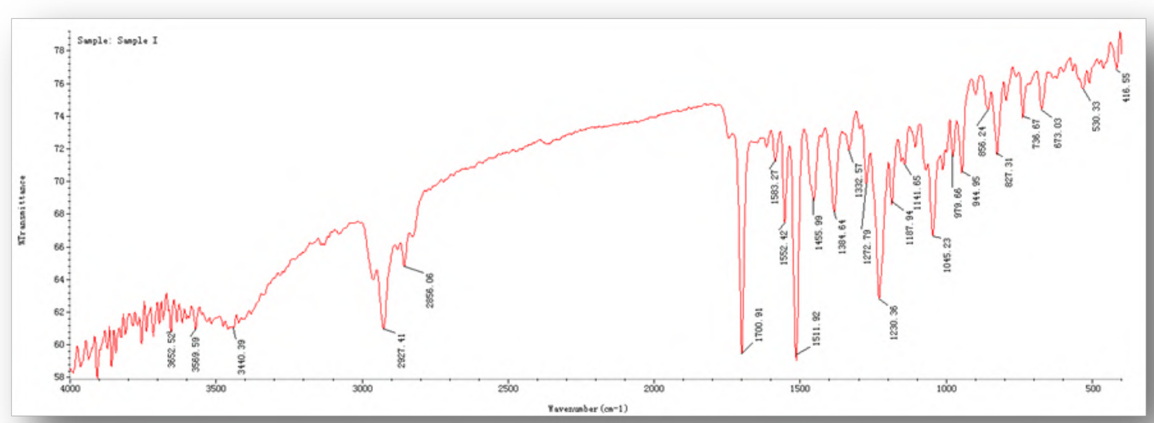


Fig 6: IR spectrum of Itraconazole.

Physical Examination

All ME-based gel formulations were white/buff thick creamy preparations with a smooth uniform texture and a glossy appearance.[33]

Drug content

Drug content was calculated using the equation, which was obtained by linear regression analysis of calibration curve of itraconazole in methanol. The drug content of all ME gel formulation was found to be 94-104%.[33]

Determination of pH

The pH values of microemulsions were varied from the range 6.09 to 6.34 which lies in the normal pH range of the skin.[34]

Spredability study

The Spreadability numbers suggested that the emulgel could be easily distributed with a minimal degree of shear. The spreadability of the gel is critical for patient compliance and aids in uniform application of the gel to the skin. A good gel will spread quickly and have a wide spreadability. ME gels prepared with low concentration of carbopol F1 belonged to fluid gel category, having more spreadability values. The stiff and semi stiff formulations were made with increasing concentrations of carbopol and xanthan gum, while the formulations F3 and F6 made with 1.5 g of carbopol were stiff and semi stiff. 1.5 g xanthan gum was classified as very stiff. The spreadability of formulations reduces as the concentration of gelling ingredient in the formulation increases.

Table 5: Spreadability studies.

Formulation Code	Drug content	pH	Spreadability gm.cm/sec
F1	102±0.14	6.1±0.69	1.06±0.2
F2	99±0.75	6.09±0.70	0.83±0.1
F3	103±0.25	6.11±0.57	0.56±0.12
F4	102±0.14	6.34±0.28	0.81±0.13
F5	100±0.15	6.19±0.35	0.76±0.17
F6	98±1.86	6.31±0.19	0.63±0.2
F7	101±0.12	6.14±0.29	0.96±0.14
F8	94±0.54	6.10±0.66	0.85±0.16
F9	99±0.6	6.12±0.48	0.96±0.10

Viscosity study

The viscosity results helped to understand the influence of various formulation parameters on consistency, spreadability and drug release. Generally consistency of formulations depends on the ratio of solid fraction to liquid fraction which produces structure.

The viscosities of ME based gels of itraconazole at low and high shear rate are given in table. Formulation containing CBP (F1-F3) exhibited high viscosity than other formulations. This is due to difference in the type of gelling agent which results in changing the structure consistency and low hygroscopicity of XG and mixture of polymers (CBP:XG), (CBP:CMC), (CBP:CMTG) (1:1) ratio as compared to CBP 934. Shear thinning was observed in all created formulations, as the viscosity was found to be reduced as the shear rate was increased (Table). Shear thinning occurs when shear is applied and the structure begins to break down when the sites of contact are disturbed and the polymeric chain aligns.Shear thinning

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behaviour is a desirable property for the topically applied preparations. Since, all prepared formulations showed pseudoplastic behaviour indicates good spreadability.

Table 6: Viscosities of ME based gels of itraconazole.

Formulation code	η* max (cP)	η** min (cP)
F1	350.47	224.36
F2	1588.24	680.96
F3	1493.45	418.39
F4	58.67	15.6
F5	1063.92	223.44
F6	1543.49	304.62
F7	1047.47	417.28
F8	868.69	332.65
F9	776.43	292.85

*Viscosity at high shear rate (100 rpm); **Viscosity at low shear rate (11.5 rpm).

In vitro drug release

All the batches of itraconazole ME gels showed drug diffusion within the range of 58.57±1.48% to 96.66±1.89% at the end of 6h.

The (CBP: CMTG) (1:1) containing gels showed maximum 96.66±1.89% drug release at the end of 6h. (CBP: CMTG) (1:1) gels exhibited higher drug release in comparison with gels formulated with CBP, XG and mixture of polymers (CBP: XG), (CBP: CMC) (1:1) ratio. As the concentration of CBP was increased in formulations (F1-F3) drug release was found to be decreased. This may be attributed to increased viscosity of carbopol gels.

Due to the difference in viscosity of the polymers, when the concentration of gelling agents in formulations increases, the diffusion of formulations reduces.

The in-vitro release of prepared formulation compared with marketed formulation (Itratrox gel 1% w/w). From the comparison it was observed that formulation F9 shows 96.66±1.89% drug release at the end of 6h and marketed Itratrox gel (1% w/w) shows 90.56±1.75% drug release at the end of 6h. From the result it was observed that ITZ ME gel of F9 batch shows more drug release compared to the marketed formulation.

Table 7: Formulation drug release percentages in Phosphate Buffer (Ph 7.4) for Formulation batches F1-F6.

Time (hr)	F1	F2	F3	F4	F5	F6
0	0.00	0.00	0.00	0.00	0.00	0.00
0.5	11.95±1.09	1.9±2.56	1.6±3.17	4.6±3.17	2.28±1.22	1.80±1.26
1	18.09±0.93	10±1.85	2.80±1.17	14.80±1.17	6.47±1.32	3.90±2.69
2	23.80±1.52	17.14±1.62	6.61±0.91	28.61±0.91	16.85±1.56	8.90±2.17
3	34.85±1.75	30.47±1.43	12.61±1.31	47.61±1.31	29.09±1.23	15.42±0.67
4	52.85±2.2	43.33±1.58	27.61±1.63	57.61±1.63	46.23±2.10	27.46±1.84
5	64.23±1.21	56.19±1.28	41.80±1.56	64.80±1.56	58.09±2.30	42.19±1.04
6	71.21±1.13	66.66±1.89	58.57±1.48	78.57±1.48	75.23±1.56	69.52±1.42

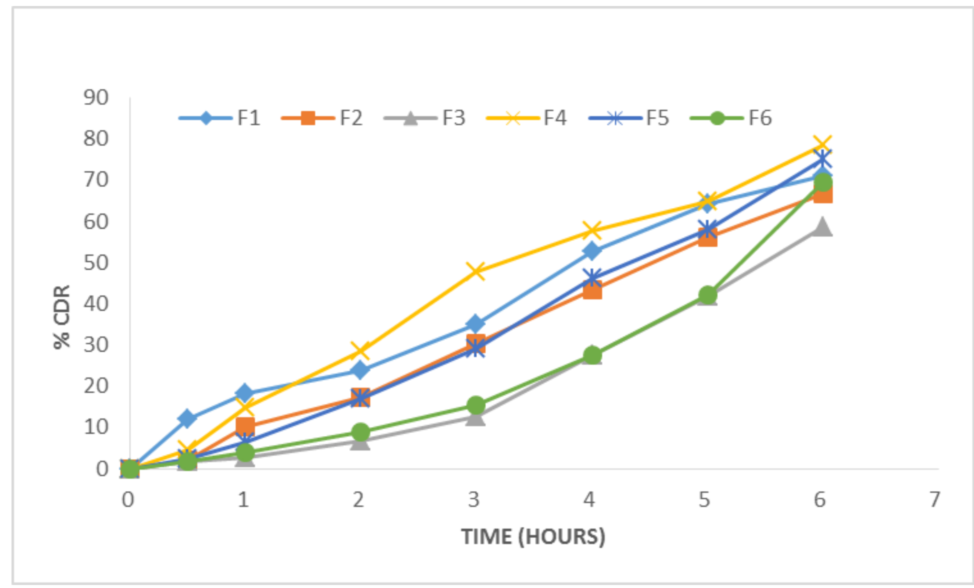

Figure 7: Formulation batch F1-F6 percentage medication release in Phosphate Buffer (Ph 7.4).

Table 8: Formulation drug release percentages in Phosphate Buffer (Ph 7.4) for Formulation batches F7-F9.

Time (hr)	F7	F8	F9	Standard
0	0.00	0.00	0.00	0.00
0.5	4.28±2.31	6.28±1.91	6.0±2.56	5.18±1.58
1	13.42±1.91	23.18±1.22	24±1.85	20.46±1.20
2	24.47±1.13	36.90±1.12	37.14±1.62	34.40±1.56
3	28.52±1.59	40.76±1.49	60.47±1.43	54.80±1.40
4	46.33±1.87	52.38±1.13	83.33±1.58	72.62±1.90
5	62.57±1.65	75.66±1.94	86.19±1.28	81.72±1.48
6	67.61±2.60	82.85±1.16	96.66±1.89	90.56±1.88

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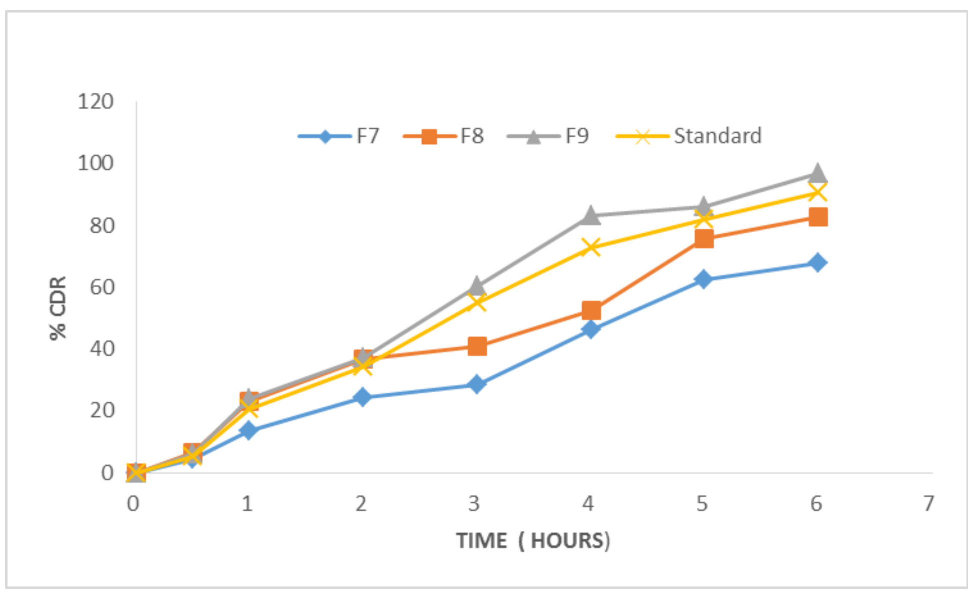

Figure 8: Percentage Drug Release of Formulations in Phosphate Buffer (Ph 7.4) for Formulation batch F7-F9.

4. CONCLUSION

ME gel produced with oil (5%), S/Cos (45%), water (50%) and (CBP: CMTG) (1:1) outperformed all other formulations in terms of overall formulation quality. Developed microemulsion system provides solubilization of hydrophobic drug, thus impart availability of itraconazole in formulation, where as globule size and zeta potential was 885.5dnm and-0.118, respectively, indicating the stability and proper formulation of microemulsion. The prepared ME gel can be considered as cost effective formulation because of reduction of topical dose of itraconazole in formulation. The F9 batch had the highest release (96.66±1.89). The prepared microemulsion gel show better release profile than marketed preparation. Furthermore, they were shown to have a better permeation and look.. It was a shear thinning system because all formulations exhibited non-Newtonian pseudoplastic behaviour. Thus, the results of this research study clearly indicated a promising potential of the itraconazole ME gel as an alternative to the conventional dosage forms.So itraconazole ME gel can be used as an anti- fungal agent for topical drug delivery.

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6. CONFLICT OF INTEREST

All authors approve the final manuscript and declare that there are no conflict of interests.

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Microneedle: A Revolution in Transdermal Drug Delivery System

Yogeshwari Rajendra Deshmukh*, Akshay Popat Jagadale, Bharatee Pandurang Chaudhari, Vivekkumar K. Redasani

Department of Pharmaceutics,
YSPM's Yashoda Technical Campus, Faculty of pharmacy,
Wadhe, Satara, India.

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ABSTRACT

Hypodermic needles, topical lotions, and transdermal patches are the most often used modalities for transdermal medication delivery. Because the stratum corneum layer of the skin acts as a barrier for molecules, only a few molecules are able to reach the site of action. Many decades ago, a new type of delivery device known as microneedles was originally envisaged, addressing shortages and keeping the advantages of hypodermic needle and traditional transdermal drug-delivery methods to some extent. The basic concept is to disturb the skin layer, resulting in micron-sized channels that lead the medicine straight to the epidermis or higher dermis, from whence the treatment can reach the systemic circulation without passing through the barrier. This review discusses the numerous possibilities and uses of microneedles. Microneedles of many sorts can be manufactured, including solid, dissolving, hydrogel, coated, and hollow microneedles. The fabrication process chosen is determined on the kind and material of the microneedle. In addition, they outline the assessment test for the same. This technique is now being used in a variety of sectors, including oligonucleotide distribution, vaccine distribution, insulin distribution, and even cosmetics. Many microneedle devices have entered the market in recent years. Although further study is required to tackle the different hurdles before the microneedles may effectively enter the market.

INTRODUCTION-:

Hypodermic needles and topical creams are most commonly used when it comes to delivery of the drug through the skin. Needles are less accepted by patients due to pain associated with them and topical creams show less bioavailability. Skin serves as the major barrier for delivering drug through the topical route. Skin is made up of three main layers-the outermost stratum corneum, middle epidermis and the thickest of all, dermis. The stratum corneum layer behaves like a major barrier as it allows only certain molecules like lipophilic and low molecular weight drugs to pass through it. The relatively less permeability of the layer presents many problems in designing topical formulation [5,6]. Various topical or transdermal delivery systems have been investigated for improving drug permeation through the skin like nanocarrier loaded topical creams, transdermal patches, and microneedles[7,8]. These structures are used to pierce the skin's upper layer in order to improve transdermal drug distribution by allowing the transport of a variety of molecules that cannot be delivered through the skin through passive diffusion alone[10]. Since the size of these microchannels is in microns and the maximum dimension of standard macromolecules delivered into the body is in nanometers [11]. Microneedles may be used to transport macromolecules such as insulin, growth hormones, immunobiologicals, proteins, and peptides. Microneedle innovations are classified into four types: solid microneedles for skin pretreatment to improve skin permeability, drug-coated microneedles, polymer microneedles that encapsulate drugs and completely or partially dissolve in the skin, and hollow microneedles for drug injection into the skin[13,14]. Microneedles are most widely used for the transdermal delivery of drugs and vaccines that require prolonged exposure, with dissolving and biodegradable microneedle technologies being the most popular.

Skin microanatomy

The biggest human organ and the body's first natural barrier is the skin (cutis). It has a surface area of around 2 m^2 and is 102–104 times less permeable than a blood capillary wall, accounting for about 15% of an adult's overall body weight [15]. The epidermis, dermis, and subcutaneous layers are the three histological layers of the skin that are usually shown in contrast to tissue layers [15]. In humans, the outer epidermis, a 5-layered assembly of keratinocytes (95 percent of cells), is usually 0.02–0.2 mm thick and 50–150 m thin. The dead skin layer, also known as the stratum corneum (SC), is the epidermis' outermost layer and is primarily responsible for the skin's physical properties due to its "brick and mortar"

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construction [16]. The hydrated keratin corneocytes are the "bricks" trapped in a "mortar" with several lipid bilayers of ceramides, fatty acids, cholesterol, and cholesterol esters [17]. The dermis is a layer underneath the epidermis and it is much thicker than the epidermis (normally 2–4 mm dense) and includes collagen (70 wt %), certain immunologically active cells, connective tissues, blood and lymph vessels, glands, hair follicles, and nerve endings [18]. The hypodermis, or subcutaneous membrane, is the innermost layer of the skin that lies under the dermis and is mostly made up of adipose tissue (fat). The dynamic capillary network in the dermis and hypodermis is important for transdermal systemic transmission. Despite the fact that it is easy, overcoming these obstructions is quite complex, especially peptides and proteins these are bigger molecules. Many drugs diffuse slowly across the skin, with lead times measured in hours before reaching steady-state fluxes. As a result, reaching therapeutically successful drug levels without increasing skin permeation is difficult. The techniques to penetrate the SC's permeability layer have recently been the subject of extensive study in a controlled and reversible manner. This would potentially increase the number of drugs that can be administered through the skin [19]. In recent years, some physio-chemical approaches to undermine the epidermis and thereby improve transdermal transmission have been developed. Chemical enhancers to MN technology, electrophoresis, iontophoresis, sonophoresis, laser therapy, and synergistic combinations of two or more pathways are one of the methods. MN arrays made from different metallic and polymeric materials have recently been the primary subject and are being extensively researched by the science community. By only reaching the top layer of skin and delivering molecules through the skin membrane, these micron-sized instruments aim to reduce the discomfort associated with hypodermic needle injections.

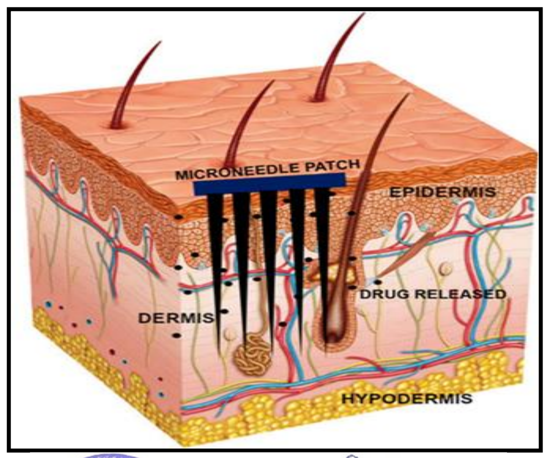

Fig. no 1: Anatomy of skin anatomy

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1. Benefits and limitations

Benefits

- Reduction in dosing frequency leads to better patient compliance
- Non-invasive delivery
- Avoidance of first pass metabolism
- Avoid gastrointestinal incompatibility
- No side effects
- Maintain plasma drug level
- Improved bioavailability
- Easy administration of large size molecules
- Suitable for drug with short biological half life and narrow therapeutic index

Limitations

- The dose accuracy is less as compared to hypodermic needle
- The thickness of the SC varies from person to person, so skin penetration varies.
- Variation of delivery due to environmental factors such as weather and skin condition
- The risk of veins collapsing as a result of repeated injections
- In the case of hollow and solid MNs, the tips can break and stay inside the skin.

2. Mechanism of Microneedle

The diffusion mechanism is used to administer the drug through the topical pathway. The skin is briefly damaged during the microneedle drug delivery system. A microneedle implant is created by arranging hundreds of microneedles in clusters on a tiny patch (similar to a standard transdermal patch present in the market) in order to provide a sufficient amount of drug to provide the desired therapeutic reaction. It pierces the stratum corneum, allowing it to bypass the boundary layer. The drug is injected directly into the epidermis or upper dermis layer, where it enters the systemic circulation and produces a therapeutic reaction until it reaches the site of action [6, 7]. Figure 2 illustrates the mechanism of drug delivery through microneedles.

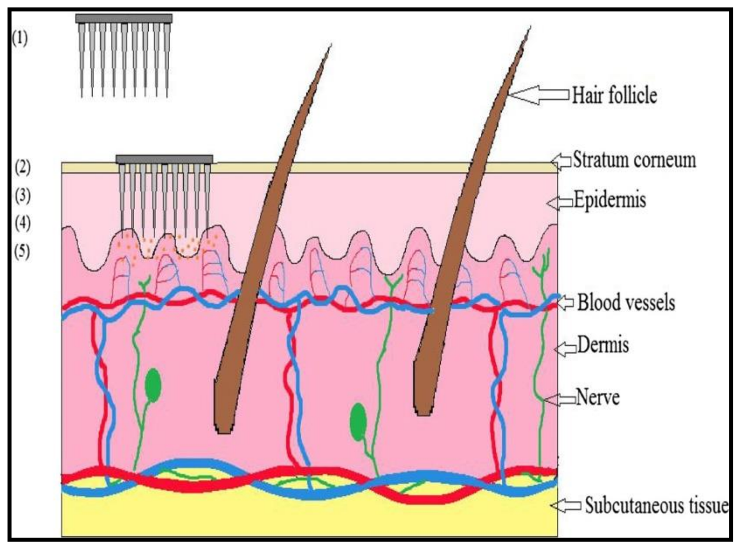

Fig.no 2 Mechanism of drug delivery by microneedle device: (1) Microneedle device with drug (2) Device implanted; (3) Temporary mechanical destruction of the skin; (4) Drug release in the epidermis; (5) Transport of the drug to the sites of action

3. Fabrication material

Microneedls may be made out of a wide range of materials, including metals and polymers. The materials used in the manufacture of microneedls should have properties including such durability, economy, user friendliness, accessibility, high tensile strength, corrosion resistance, mechanical stability, and so on. Microneedls would be more acceptable and useful as a result of this [20].

3.1 Silicon

In the 1990s, silicon was used to build the first microneedle [21]. Silicon has a crystalline structure and is anisotropic in nature. Its properties are determined by the crystal lattice's orientation, which has various elastic moduli (50 to 180 GPa) [22, 23]. Its adaptability allows it to produce needles in various sizes and shapes. It is a flexible substance due to its appealing physical properties.

Silicon-based microneedles have a high mechanical force, allowing them to penetrate the flesh [24]. The needle material and geometry play a role in the silicon microneedle fabrication process. A silicon dry-etching process based on reactive ion etching with a chromium mask was used to create short silicon microneedles. Furthermore, it was the first

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material chosen for the manufacture of microneedles. Silicon has played an important part in the construction of microstructures and micro-electro mechanical systems (MEMS).

3.2 Metals

Stainless steel and titanium are the most common metals used. Often used are palladium, copper, and palladium-cobalt alloys. They have excellent mechanical and biocompatibility properties. Metals are more ideal for microneedle processing than silicon because they are solid enough to prevent cracking. Stainless steel was the first metal used in the manufacture of microneedles. Titanium is a suitable stainless steel substitute [25].

3.3 Ceramics

Chemical resistance is the primary reason for the use of alumina (Al2O3). Because of the strongly energetic ionic and covalent bonds within Al and O atoms, it forms a solid oxide. Calcium sulphate dihydrate [Gypsum (CaSO4 0.2H2O)] and calcium phosphate dihydrate [Brushite (CaHPO4.2H2O)] are two other varieties of ceramics used. Ormocer®, a synthetic polymer ceramic, has been used in recent years. It is a cross-linked copolymer in three dimensions [26]. Using various organic units throughout polymerization can result in a polymer with various properties. They are often made using a micromolding technique. A micro-mold is filled with ceramic slurry. Micro-moulding methods are less expensive and have the ability for scale-up [8].

3.4 Glass

Glass is used to make geometrically variable microneedles on a small scale. Despite its brittle appearance, silica glass is commonly used for microneedle preparation. Furthermore, since borosilicate glass is now more elastic, it can be made by hand. As a result, the use of glass as an industrial fabricating medium for microneedles is limited [25].

3.5 Carbohydrates

Carbohydrates such as maltose are also used in the manufacture of microneedles. Microneedles are prepared using heated slurries or softens of carbs. Carbohydrates are the most cost-effective, compatible, and stable material for the production of microneedles. These properties made carbohydrate a viable alternative to silicon, plastics, polymers, and other building materials [27]. Other sugars that can be used to make microneedles include galactose, mannitol, sucrose, trehalose, and xylitol [28]. Temperature-based MN fabrication

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may also not be appropriate for thermo labile drugs or materials. As a result, it could have an effect on their storage state, potentially causing reliability problems.

3.6 Polymer

For microneedle preparation, a wide range of polymers have been identified, including polymethyl methacrylate (PMMA), polylactic acid (PLA), polylactic-co-glycolic acid (PLGA), polyglycolic acid (PGA), polycarbonate, cyclic-olefin copolymer, polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA), polystyrene (PS), polymethyl vinyl ether-comaleic anhydride, SU-8 photoresist. These polymers are used to make dissolving, biodegradable, and hydrogel-forming microneedle clusters. These polymers produce microneedles that are less strong than other materials but stronger than glass and ceramics [25].

4. Types of microneedles

Strong, polished, dissolving, hollow, and hydrogel microneedles are among the various forms of microneedles that have been fabricated and studied for use in drug distribution. In Figure no. 3, different types of microneedles are seen, each with their own set of characteristics. The drug is delivered into the epidermis in a different manner with each kind of microneedle. Some are used solely to build pores in the stratum corneum, while others are precoated with the drug solution, dissolvable, or prefilled with the drug solution [29–32].

4.1 Solid microneedle

These microneedles employ a "poke and patch" technique. It is first applied to the skin to stimulate pore creation, and then it is removed. Following that, a medication formulation is applied to the skin, which serves as an external reservoir and aids drug absorption. The ‘poke and patch' tactic is used by these microneedles. It is first applied to the skin in order to stimulate pore creation, after which it is removed. Following that, a drug formulation is applied to the skin, which serves as an external reservoir for the medication and aids in its penetration [29-30]. Li et al. investigated polylactic acid microneedles and discovered that biodegradable polymer solid microneedles may puncture the stratum corneum and improve medication absorption. Microneedles with a depth of 800 m and a density of 256 MNs/ cm^2 were shown to improve drug penetration [31].

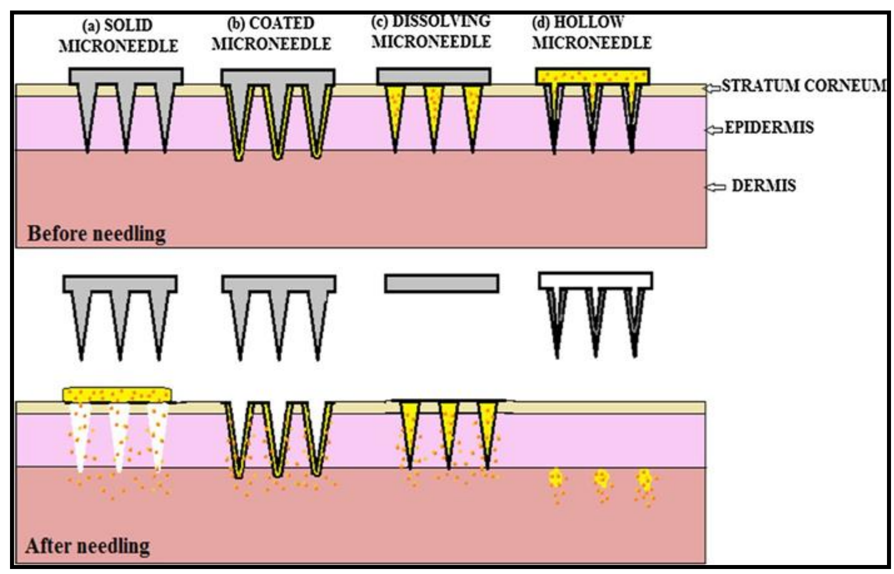

Fig no. 3 Types of microneedles (a) Solid microneedle (b) Coated microneedle (c) Dissolving microneedle (d) Hollow microneedle[25]

4.2 Coated Microneedle

The drug solution or dispersion layer is encapsulated in this type of microneedle, causing drug dissolution and fast delivery. The overall amount of drug loaded is determined by the thickness of the coating layer and the needle size. Coated microneedles have been extensively studied, particularly in the areas of medication delivery, vaccines, DNA, and biomolecules. Coated microneedles may now be used in a wide range of scientific applications because to recent developments in coating composition [32-33]. Li et al. coated each microneedle with distinct formulations and drugs, allowing numerous therapeutics with varying characteristics to be delivered simultaneously. These dyes were given in both water soluble and water insoluble forms [34]. Chen and colleagues coated PLA microneedles with sulforhodamine B and discovered that the drug delivery effectiveness was around 90%. The continuous drug delivery was validated in mice in vitro tests.

4.3 Hollow MN

Traditionally, hollow microneedles, which are similar to micron syringes, are used to inject liquid formulations into the subcutaneous layer of the skin. When compared to solid microneedles, these microneedles can deliver higher medication dosages. A empty area inside these microneedles is filled with the medication solution or dispersion. A particular medicine can be administered into the skin by injecting a liquid formulation through implanted hollow

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microneddles. Various strategies including as diffusion, pressure, and electrical aid can be used to distribute medicinal molecules continuously via these hollow microneedles.

Hollow microneedles have been made using a variety of techniques. Large batches of hollow microneedles were made using microelectromechanical systems (MEMS) technology. Yu et al. used MEMS methods to create a cylindrical hollow microneedle that went through three basic procedures: photolithography, Bosch deep reactive ion etching, and micromachining [37]. A wide range of materials, including silicon, metal, glass, ceramics, and polymers, can be used to make hollow microneedles. High molecular weight compounds like as antigens, proteins, and oligonucleotides are widely used. The likelihood of needle apertures clogging during skin piercing and resistance to flow are two key drawbacks of this approach [35-36].

4.4 Dissolving/ biodegradable Microneedle

These microneedles are typically made of a biodegradable polymer in which the medication is enclosed. When they are placed into the skin, the drug dissolves and the medication is released [38]. In comparison to other varieties, it is a one-step application technique, therefore there is no physical removal necessary. Because it is biodegradable, it is one of the most generally approved microneedles and a superior alternative for continuing therapy. Patient compliance improves as a result of bio-acceptability. The drug release time from dissolving microneedles spans from hours to days, and it is dependent on the nature of the dissolution and the kind of polymers [39-40]. Luzuriaga et al. proposed a novel micromachining approach that uses fused deposition modelling (FDM) 3DP to swiftly design and print microneedle density, length, and form. Moreover, to increase feature size resolution, a post-fabrication chemical etching process including 3DP has been devised, with access to microneedle tip sizes as tiny as 1 mm [41].

4.5 Hydrogel forming Microneedle

It's a newer sort of microneedle that makes use of super-swelling polymers. The polymers employed in the fabrication have a higher water absorption capacity. The three-dimensional polymeric network structure absorbs the water. Because of the presence of bodily fluid, when these microneedles are put into the skin, the polymers swell. This results in the formation of conduits, which allow the drug release to reach the microcirculation from the pool. Polymer's swelling properties act as a rate-controlling membrane [20].

5. Fabrication of Microneedle

Microfabrication flourished in the mid-1990s and evolved into a variety of tiny devices, eventually leading to the invention of microneedles. The ALZA collaboration first established the notion of an microneedle, but it wasn't until 1988 that microneedles were explored in scientific studies for TDDS owing to the growth in new developing materials and development in engineering technologies. The needle geometry (size, shape, and breadth) is optimised for insertion into the skin throughout the manufacture process. The materials utilised and their application will be discussed in detail in the next section, as will the details of each methodology for the fabrication of various microneedles.

5.1 Micromolding

Micromolding procedures are the most widely documented methodology for the manufacture of microneedles. Photolithography and moulding techniques are often used to create the PDMS micromold (which is typically manufactured in one step by drilling on the surfaces of 2-mm thick PDMS sheets with a laser beam and then filling with the material such as ceramics and polymeric formulations (sugars, natural and synthetic polymers)). Although this technology enables for upscaling production, it has limits since it often entails many time-consuming procedures such as master preparation, mould creation, and plasticisation of thermoplastic polymers above their glass transition temperature, preventing the use of thermo-liable pharmaceuticals [42].

5.2 Lithography

Etching (both wet and dry) the subtractive procedure has also been described for the manufacture of microneedles (carving a 2D substrate from a 3D structure). Lithography and wet etching are two typical methods for producing silicon and glass microneedles.

The disadvantages of such approaches include the requirement for specialised equipment in clean rooms and the generation of hazardous waste, which is costly, difficult, inconvenient, and harmful to the environment. The benefits of adopting additive technologies for microneedle production, such as drawing lithography, include the ability to manipulate the size and form of microneedles to increase mechanical stability, release kinetics and release profile. However, utilising photocurable polymers can be problematic since UV light has the potential to inactivate the drug and leave harmful photo-initiator residue in the final microneedle. This process has been improved by employing an in situ lens-based lithographic

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method to transform the tapered cone generated into bevelled, chisel tip microneedles using microelectromechanical masking and etching [43]. 3D polymer microneedle structures may be created from 2D surfaces or droplets using additive methods like as 3D printing, droplet born air blowing, electro-drawing, and thermal drawing [44].

5.3 3D Printing

3D printing, which first appeared in the 1980s, is a family of technologies that uses computer-aided design (CAD) models to construct a real thing through the fabrication of layers. It has subsequently revolutionized the pharmaceutical industry. This is a one-step procedure that enables for the quick manufacture of microneedles with a wide range of geometries while maintaining a high degree of consistency and complexity.

Stereolithography is one of the technologies that makes up 3D printing (for anticancer therapy, insulin skin delivery as well as delivery of model dye) [45]. Digital light processing (DLP) photopolymerisation-based techniques that allow the production of structures by layer wise polymerisation of UV sensitive polymers via a heating process (photopolymerisation) and Two-Photon Polymerisation (transdermal delivery of polymer-ceramic hybrid materials) [46].

Stereolithography has been widely used as a method of manufacturing MNs in recent years. Fatouros et al. developed successful in vitro TDD utilising stereolithography-printed 3D MNs. Mechanical strength, insertion force, and visualisation investigations were performed on these polymer-based MNs. This study found that these 3D printed MN arrays had good penetration in human skin and significantly increased dye transport across human skin [45]. This demonstrates the enormous potential for 3D printed MN systems in transdermal medication administration.

5.4 Electro-drawing

The electro-drawing approach is a non-contact, low-temperature, UV-free way of producing PLGA microneedles. This method yielded MN arrays with optimized form and size. This approach employs a pyroelectric field and micrometric elements to regulate temperature on a microscale, as well as a pyroelectric crystal to regulate electrohydrodynamic activity once a voltage is introduced to the circuit [47]. 3D printing is a versatile technology that allows for personalization and customization; however, the resolution is restricted, hence the length of the resulting microneedles is measured in millimetres with tip diameters of 100 m. Droplet-

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born air blowing offers improved fabrication conditions without the use of heat or UV irradiation, as well as high productivity and has been commercialized [48].

5.5 Thermal Drawing

Thermal drawing includes heating a polymer that is vertically pulled at a regulated pace by a metal pillar arrangement. After cooling, the neck is shattered by quick drawing, generating the microneedle structure. However, there are concerns with the fracture stage, which results in a flat or extended apex, restricting insertion capabilities as well as the tip. Despite the fact that it is a very basic procedure, optimization has proven difficult, therefore other fabrications have been examined in recent years [49].

5.6 Magnetorheological Drawing Lithography

Recently, magnetorheological drawing lithography has been examined as a unique approach for the efficient production of microneedles, bio-inspired microneedles, and microneedle arrays. This additive process swiftly forms a 3D microneedle structure from a droplet of curable magnetorheological fluid extracted straight from any substrate to build a 3D microneedle under a magnetic field. This approach combines the benefits of thermal drawing (without the requirement for a mask or UV irradiation) with the benefit of not requiring temperature modifications throughout the drawing process. These microneedles demonstrated the versatility and viability of the magnetorheological drawing lithography (MRDL) technology [50].

5.7 Droplet Air Born Blowing

Kim et al. investigated the droplet air born (DAB) blowing process and discovered that it provides gentle (4–25 degrees) and rapid (less than 10 minutes) microneedle fabrication while minimising drug loss. The drug content can also be regulated by the droplet dispenser's pressure and time, and also the air blowing shapes the droplet to the microneedle with enough force to penetrate the skin. Insulin DMNs based on DAB have shown promise in providing complete drug delivery with no waste [51]. To make DMNs and test the activity of encapsulated drugs, this method was combined with centrifugal lithography (two droplet DAB). It was reported that the centrifugal lithography manufacturing process for DMNs put less stress on the drug-loaded DMNs, reducing action loss over time, proving centrifugal lithography's efficacy in DMN fabrication [52]. Kim et al. studies of allergen (Dermatophagoides farinae (D. farinae) extract (DFE) loaded MNs (fabricated using DAB)

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for allergen specific immunotherapy (SIT) and atopic dermatitis revealed stable allergenicity at 10 g of DfE sufficient to elicit immunogenic responses all without side effects when compared to 100 g of subcutaneous injection. Each droplet was air blown and strengthened to form an microneedle, so DAB was used. The conditions are also less harsh, removing the need for heat/UV irradiation. It was also feasible to load each microneedle without loss by design, and manufacture was completed in 10 minutes [53].

6. Evaluation of microneedles

6.1 Characterization methods

The medicine can be placed onto or into the microneedles in two forms: suspension/dispersion or encapsulated (liposomes, nanoparticles, nanoliposomes). The polymer solution can be applied to the medication as a coating or as a patch. Depending on the kind of formulation utilized in the microneedles, several physicochemical characterizations such as particle size, polydispersity index, viscosity, and zeta potential can be examined for loaded drug [55]. For a patch that is applied following pre-treatment, drug release, adhesion, and penetration tests are done. Dynamic light scattering, X-ray scattering, and transmission electron microscopy can be used to determine the size, internal structure, and crystallinity of liposomes or nanocarriers. Drug dispersion and microneedle stability may be examined at various temperatures, pH levels, and simulate in-vivo physiological settings (cell line or tissues). Other testing, including as solubility studies, drug content, in-vitro release testing, and biocompatibility studies, are also carried out on microneedle designs [26,54].

6.2 Dimensional evaluation

To analyze the needle geometry and quantify the tip radius, length, and height of the microneedle, many approaches are utilized. Optical or electrical microscopy is the most often used approaches. The analysis of a 3D image provides a more accurate view of needle shape and aids in quality control. This was accomplished using a scanning electron microscope (SEM) and a confocal laser microscope. SEM creates a picture of a sample by scanning it with a focused stream of electrons that interact with the atoms in the sample and produce different signals that provide information about the sample's surface topography and composition. High-resolution pictures are produced using confocal laser microscopes [55,56].

6.3 Mechanical properties or insertion forces

A microneedle must be sharp and slim enough to easily penetrate the skin while also being strong enough not to break once inside the skin. Table 3 lists the mechanical tests performed on microneedles. The force at which the microneedle ends up losing structural integrity and the insertion depth are two critical factors in the safe and efficient design of microneedles. The safety factor' is the ratio of these two forces. The ratio should be as high as feasible [57].

Table no.1 Mechanical characterization studies

Parameter	Test
Insertion force	Dye making, force displacement test or electrical measurement
Insertion depth	Histological cryosectioning and staining, confocal microscopy and optical microscopy
Failure force	Pressing a device on a rigid surface, displacement force tests

6.4 *In-vitro* skin permeation studies

The diffusion cell apparatus is used to determine drug permeation through the skin. The experiment primarily employs pig ear skin, which is mounted between the receptor and donor compartments. The cumulative permeation profiles of microneedle treated and untreated skin are compared [58].

6.5 *In-vivo* animal model studies

The study can make use of hairless rats. To anaesthetize the animal, a suitable technique must be used. Trans-epidermal water loss (TEWL), which is measured before and after microneedling, is one of the parameters considered. This parameter is measured using a Delfin Vapometer [59].

7. Commercialization of Microneedle product

7.1 FDA regulatory requirements for commercialization

Because microneedle technology is a comparatively unique and modern field, no completely separate regulatory requirements for microneedle-based products have been established to date. Traditional transdermal patches are only applied to the skin's surface, whereas microneedles penetrate the stratum corneum barrier and, in some cases, invade into the viable

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epidermis and dermis. Interrupting the skin's defensive layer is a completely different mechanism of action, which prompts the emergence of new scientific/regulatory demands. As a result, in addition to the well-defined requirements for the pre-existing transdermal patch systems, new regulatory specifications for microneedle systems should be defined. Some of the major regulatory issues that must be addressed when planning for the commercialization of microneedle devices are as follows [22]:

1. Needle characteristics such as materials, length, adjustability, sharpness, and geometry must be carefully designed.

2. Microneedle devices should maintain adequate microbiological standards.

3. The content of the Microneedle systems should be consistent.

4. High-quality manufacturing techniques, as well as safe and secure packaging, should be used.

5. To avoid the possibility of re-use by patients or others, Microneedle systems made of non-biodegradable materials may require a self-disabling mechanism to ensure a single-use only. Furthermore, safe and non-hazardous disposal procedures for these Microneeedle systems should be defined.

6. If the Microneedle device is reusable, cleaning or disinfection instructions should be included.

7. The issue of safe Microneedle material deposition in the skin without causing adverse skin reactions should be addressed, particularly for microneedle products intended for long-term use.

8. The proposed labelling for the microneedle device, including package labelling and usage instructions, should be provided.

9. Microneedle systems should be simple to use and produce repeatable results without complications. They should also be used in conjunction with the appropriate application device to ensure proper insertion and pain-free delivery.

10. Immunological safety assurance for the microneedle systems may be required.

11. For microneedles requiring intermittent and repeated applications, the long-term safety profile of microneedle application should be discussed.

7.2 Approved products

Derma roller was the first microneedle product. Many microneedle products are now available on the market that have been cleared for medical and cosmetic use. Table 4 contains a list of some of them. Many companies in Germany, the United States, Europe, and Japan sell microneedle products [59,60, 61].

Table no. 2 Approved microneeedle products

Product name	Company name	Product description	Use
Dermaroller®	Dermaroller® Germany,White Lotus	A cylindrical roller with solid or metal microneedles ranging in length from 0.2 to 2.5 mm.	Enhance skin texture, heal scars, and cure hyperpigmentation.
C-8 (Cosmetic type)	The Dermaroller Series by Anastassakis K.	A needle length of only 0.13 mm (130 m) is used.	Used to improve the penetration of topical agents.
MicroHyala®	CosMed transdermal drug delivery	Dissolving microneedle patch with hyaluronic acid	Wrinkle treatment
Macroflux®	Alza/Johnson and Johnson	Coated titanium microprojections	Biopharmaceutical delivery has improved.
Soluvia®	Sanofi Pasteur Europe	A syringe is attached to a hollow microneedle.	Influenza vaccination
h-patch	Valeritas	A small adhesive machine, similar to a patch, is used.	Drug delivery in subcutaneous tissue (insulin)
Microstructured transdermal system	3M	Hollow microneedle	Biologics and other small molecules will be delivered.
MicronJet®	NanoPass Technologies	4 hollow silicon needles with a length of less than 500μm	Used to deliver influenza vaccine.

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8. Status of US patents[20]

Patents encourage the use of microneedles as a general-purpose application tool while also integrating research in their respective fields. The authors outlined patents from the previous 12 years (2005–2017) in this review. So far, many researchers have concentrated on approaches that have been developed as novel processes in the fabrication of microneedles. The importance of collecting patent data is to provide an update and overview of current trends and future aspects of microneedles. As a result, the United States (U.S.) has the world's largest pharmaceutical market and the greatest number of patents in terms of microneedles [62]. As a result, the authors retrieved relevant patents granted in the United States that were related to microneedles. This information was gathered and summarized in Table 3 [63, 64].

Table no. 3 US Patent Status in US

Sr.no.	Title of patent	Date	Patent no	Name of inventor
1	Metallic microneedles	13 June 2017	US9675790	Stoeber et al.
2.	Surface micro machined microneedles	23 may 2006	US7048723	Frazier et al.
3.	Method for fabricating microneedles	29 August 2006	US7097776	Govinda Raju
4.	Microneedles and microneedles fabrication	3 March 2009	US7497980	Xu et al.
5.	System and method for drug delivery and microfluidic application using microneedles	14 July 2009	US7560036	Golubovie-Liakopoulos et al.
6.	Molecular sieve and zeolite microneedles and preparation	20 April 2010	US7699819	Yeung et al.
7.	Apparatus and method for manufacturing microneedles	04 May 2010	US7708544	Pricone

8.	Method and/or apparatus for puncturing a surface for extraction, in situ analysis, and/or substance delivery using microneedles	13 July 2010	US7753888	Mukerje et al.
9.	Microneedles and methods of fabricating	31 August 2010	US1185459	Raju et al.
10.	Mechanically robust fast-dissolving microneedles for transdermal drug and vaccine delivery	27 December 2016	US9526884	Yan et al.
11.	Use of cannabidiol prodrugs in topical and transdermal administration with microneedles	03 January 2017	US9533942	Stinchcomb et al.

9. Applications:

Microneedles are a relatively new biomedical advancement with non-invasiveness, high selectivity, and flexibility in use as a therapeutic and diagnostic tool. Furthermore, existing research on microneedles has expanded the range of its formulation with a variety of applications. Microneedles are a relatively new bioengineering innovation with non-invasiveness, high selectivity, and flexibility in use as a therapeutic and diagnostic tool. Furthermore, existing research on microneedles has expanded the range of its formulation with a variety of applications [65, 66]. The most common applications of microneedles are allergy diagnosis, animal identification, blood extraction, cancer therapy, cell surgery, dentistry, drug delivery, fluid sampling, gene delivery, ink-jet printing, micro-dialysis, sensing electrodes, skin treatment, vaccination, and so on.

Therapeutic Applications

Antiglaucoma

Kim et al. introduced drugs into the supraciliary space using hollow microneedles. They demonstrate significant dose sparing of antiglaucoma agents when compared to eye drops.

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This method of targeted delivery improves safety, reduces side effects, and allows for a single injection with enough drug for long-term sustained delivery [67]. Ocular drug delivery with microneedles is safe, simple, and effective. However, the limited drug carrying capacity of devices demonstrated thus far may limit clinical interpretation potential. Omid et al. created microneedles that act as aquifers for passive delivery; the capacity of the microneedles can be up to five times that of solid microneedles [68].

Diagnostics

It is impossible to create an effective therapy without a proper diagnosis. This is fundamental and necessary for the success of therapy; thereby, the use of microneedles has aided in this field. Sun et al. invented microneedles that can be used to withdraw protein antigens and therapeutic proteins in the skin for allergen physical examination or immunotherapy [69]. Skoog et al., on the other hand, developed in vivo biosensors that provides the control real-time detection of biomolecules for patient surveillance. They created nitrogen ultra-nanocrystalline diamond coated titanium alloy microneedle arrays that can predict dopamine and uric acid electrochemically [70].

Cancer diagnosis

Keum et al. developed a dual-diagnostic system that combines high-resolution imaging with electrical real-time detection of nitric oxide released from cancer tissues using an endo-microscope and microneedle sensor. This system can be used in biomedical applications to detect cancer in a simple, quick, and accurate manner [71]. In opposed to previous endomicroscopy, which can only identify microscopic pathological characteristics and frequently necessitates biopsy sampling of suspicious lesions for additional histopathological examination of cancers.

Biomarkers

Li et al. demonstrated that surface-modified microneedle arrays could quantify biomarkers in the upper dermis after laser treatment in a reliable and timely manner. It could be done safely by briefly irradiating the microneedle array application site with a laser. As IgG can be measured using this noninvasive procedure, the assay was not affected by the length of the microneedles or molecular mass [72-73].

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Other applications are being monitored, such as electrocardiography (ECG), electromyography (EMG), and electroencephalography (EEG), all of which are critical for understanding pathological and physiological conditions in humans. The electrodes are currently utilised, although they have drawbacks that can lead to incorrect results if not correctly placed and necessitate the use of gel. Renxin et al. enhanced the microneedles for EEG monitoring. Parylene-based microneedle electrode arrays (MNEAs) were used as dry electrodes capable of EEG monitoring without skin corrosion and gel. In the study, they create a flexible MNEA that can be adapted to the skin, providing not only conformal but also robust contact in comparison to conventional devices [74].

Vaccine therapy

A vaccination is a biological product. It offers active acquired immunity to a specific illness. Vaccine is a destroyed or weakened version of a disease-causing microorganism, one of its toxins, or one of its surface proteins. Vaccine treatment boosts the body's immune system and protects against future microorganism encounters. The microneedle technique has been shown to be efficient in vaccination treatment [60, 75]. A microneedle was used to administer the DNA vaccination. Immune responses seen were far superior to those obtained with standard doses [76]. An effort was also made to construct a microneedle patch for delivering influenza vaccination [77]. When the medicine is delivered by hollow microneedles rather than intramuscular injection, a lower dosage is required. The use of hollow microneedles to administer anthrax and rabies vaccines was also investigated [22]. Ogai and colleagues created hollow microneedles out of poly-glycolic acid to improve intradermal vaccination effectiveness. The precise administration of the medication in the upper dermis boosts immunity. On the 15th day after immunisation, intradermal immunisation with microneedles resulted in considerably greater antibody titers than subcutaneous injection [78]. The use of dissolving microneedles for intradermal immunisation was also examined [79]. The continued use of MNs may have a positive impact on vaccination programmes across the world. The following are some relevant examples [20]:

Table no. 4 MNs influence on immunization

Animal	Virus	Bacteria
Mouse	Chikungunya, hepatitis B, Hepatitis C	Plague, Tetanus.
Rat	Measles	-
Guinea pig	Influenza	Tuberculosis
Rabbit	-	Anthrax
Pig	Hepatitis B	-
Macaque	Japanese encephalitis, Measles	-
Human	Influenza, Rabies, Polio	-

Ocular delivery

Targeted medication delivery can be used to treat a wide range of posterior segment conditions. Nanoparticles were delivered through the suprachoroidal space via iontophoresis. The particles were observed to localize at the injection site in the absence of iontophoresis. And over 30% of nanoparticles were transported to the posterior portion of the eye when coupled with microneedles [80].

Pain therapy

Polydimethylsiloxane moulds were used to create meloxicam-loaded polymeric microneedles. In-vitro penetration investigations revealed that about 100 percent of the medication was released in 60 minutes. The drug deposition rate was determined to be 63.37 percent, with an enhanced transdermal flux of 1.60 g/cm2 /hr. When compared to a free drug solution, penetration increased 2.58 times [81]. Neuropathic pain is notoriously difficult to manage. The present therapies do not give adequate pain relief and have a number of negative effects. Dissolvable microneedles were investigated for the treatment of neuropathic pain. These supplied a calcitonin gene-related peptide (CGRP) antagonist peptide that was highly specific for the receptors. There was no skin irritation or negative effects with the analgesic microneedle patch. Within 20 minutes of treatment, almost 75% of the microneedle was dissolved [82]. The successful administration of medications by microneedle has created enormous prospects for the pain management businesses.

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Cosmetological applications

In today's world, an individual's entire look is a highly crucial criterion. Many individuals regard cosmetics as a basic necessity. Microneedles are establishing their own existence in such a cosmo-techno environment. Microneedles are the most popular type of cosmetic procedure. MNs have been on the cosmetic industry for almost ten years. Typically, a device is used to pierce the skin, followed by the application of a substance to the skin's surface to enhance it. As a result, microneedles are utilized to promote collagen penetration deeper into the skin in order to protect it from micro damage. Microneedles also improves the look of the skin. Pre-treatment of microneedle with eflornithine cream improved its efficiency in preventing hair development in mice [83]. This can be really effective in the treatment of hirsutism. However, microneedles combining finasteride and minoxidil have been shown to promote hair growth in the treatment of alopecia, particularly androgenic alopecia [84]. The microneedles are said to be very good for acne, ageing, burn injury scars, skin lesions, vulgaris, and wrinkles. It is reasonable to believe that microneedle is an example of a successful and safe new therapy [83]. As a result, traditional cosmetics and topical formulations will be microneedled to improve present practice. Furthermore, hyaluronic acid microneedles are gaining favour in the treatment of fine lines and wrinkles. Hyaluronic acid injections are frequently utilized as fillers in the same [85]. Microneedles have also been used successfully to treat acne and acne scars, particularly when combined with other methods like as fractional radiofrequency, microdermabrasion, subcision, laser therapy, CO_2 fractional-laser, and chemical treatments such as acid peels or botulinum toxin an injection etc. [86].

10. Conclusion & future perspective

According to the current review, MNs can be created to distribute drugs in a smart and optimal manner. This approach may provide the necessary medication penetration and therapeutic effectiveness while causing minimal adverse effects. MN consideration in several scientific aspects gives improved direction and a revolution in the field of TDDS. Many researchers tinkered with traditional MNs in order to get the most out of them. MNs are a type of new technology that is very adaptable and may be used for both local and systemic treatments. Microneedles have been discovered to be fabricatable utilising a variety of materials and processes, as mentioned in their respective parts of the publication. These fabrication materials and processes produce MNs that are very effective, safe, and stable. Furthermore, several patents have been submitted in their respective domains, just a handful

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of which are described in the article from 2010 to 2017. This shows that MNs are a large and potentially lucrative area of intellectual property rights concern. MNs have a wide range of uses, including aesthetic, diagnostic, and medicinal purposes. The primary goal of microneedle development is to create cost-effective, user-friendly technology that will improve its application. As a result, it has the potential to be a valuable monitoring tool for both developed and developing countries. Lastly, several authorities and regulatory agencies have approved the newly designed microneedles for clinical studies. It will increase the use of microneedles in the near future. Microneedles should be optimised for safety and health concerns in order to become an option of therapy in illnesses and disorders for the benefit of humanity. Microneedles are the cutting-edge introduction of new technologies that will have a substantial influence on TDDS and medication therapy. However, because Microneedles are under clinical trials, the future seems bright for Microneedle-based delivery methods. Microneedles also have a bright future in paediatric medication delivery. Overall, the use of microneedles is a must for the existing world's horizon. This publication attempts to cover microneedle manufacturing procedures, challenges, applications, patents, clinical studies, and future prospects.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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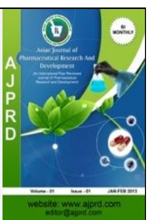

Review Article

A Review on Medicinal Plants of Natural Origin for Treatment of Polycystic Ovarian Syndrome (PCOS)

Shivani Andhalkar *[1], Vitthal Chaware [2], Vivek Redasani [3]

[1]Department of Pharmacology, YSPM's Yashoda Technical Campus Wadhe, Satara, India.
[2]Head of Department (Pharmacology), YSPM's Yashoda Technical Campus, Wadhe, Satara, India.
[3]Principal, YSPM's Yashoda Technical Campus, Wadhe, Satara, India.

ABSTRACT

Polycystic ovarian syndrome (PCOS) related infertility is a global problem that is spreading at an alarming rate. Chronic anovulation, polycystic ovaries, and hyperandrogenism, as well as abnormal menstrual cycles, hirsutism, acne, and infertility, are all symptoms of this condition. PCOS is linked to insulin resistance and elevated levels of male hormones (androgens). Among other things, an inactive lifestyle, a lack of exercise, dietary changes, and stress are all contributing factors. Curcuma longa, Aloe barbadensis**,** Mentha piperita, Allium fistulosum Cinnamonum zeylanicum, and other plants have been shown to be effective in the treatment of PCOS. The aim of this review is to summarise the most effective medicinal plants that are used in the treatment or prevention of PCOS. Special emphasis is placed on the role of insulin resistance and the possible utility of insulin sensitizers in the treatment of PCOS.

Keywords- Polycystic ovarian syndrome (PCOS), Screening methods of pcos, Pathophysiology of pcos.

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***Address for Correspondence:**

Andhalkar Shivani, Department of Pharmacology, YSPM's Yashoda Technical Campus Wadhe, Satara, India

INTRODUCTION

Leventhal and Stein in 1935 first defined a disorder, which would ultimately become known as polycystic ovary (or ovarian) syndrome (PCOS)[1]. Polycystic ovary syndrome (PCOS), a unitization of symptoms, which affects women of child-bearing age is assumptive in epidemic proportions. A resultant of imbalance in proportion of female sex hormones, results in cysts within antral follicles of ovaries. Once multiple cysts are formed in the ovarian follicles because of the hormonal imbalance, it is characterized as PCOS. Anovulation and absence of menstrual cycle prevents fertilization, and conception in women, thus pregnancy becomes troublesome[2]. PCOS affects 6–10% of women throughout the globe. According to 1990 NIH criteria 7–12 and even more individuals. According to the broader Rotterdam criteria, which makes it one of the most common human disorders and the single most common endocrinopathy in women of reproductive age.[3] The oxidative stress (OS), that will increases in inflammation, which also been reported as possible cause of PCOS[4]. Women with PCOS has several risk factors which are associated with the development of uterine cancer including fatness, hyperinsulinemia, diabetes mellitus and abnormal uterine bleeding.[5] The frequency of depression and anxiety is higher in women with PCOS than in the general population. Mood disorders are capable of impairing quality of life, which are well-known in young adult women, concerned with fertility, and in women of all ages with respect to obesity, and clinical manifestations of excessive androgen.[8]

RISK FACTORS[6]

- Obesity
- Family history of Infertility
- Family history of PCOS
- Family history of diabetes
- Fast food diet habits
- Lack of physical exercise

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PATHOPHYSIOLOGY OF PCOS[7]

The pituitary gonadotropin is fundamental to reproductive function-its production and secretion of FSH and LH is directly stimulated by hypothalamic GnRH and it is also influenced by integrated feedback mechanisms. The initial stimulus for follicular development and also granulosa cell conversion of androgens to oestrogens by stimulating the aromatase enzymes is provided by FSH. Luteinizing Hormone(LH) characteristically known for its role in the luteal phase by promoting secretion of progesterone, also it has a vital role in the follicular phase, for inducing thecal androgen production. Women with PCOS often secrete more LH and this might result in higher theca cell androgen secretion. to maintain gonadotropin secretion pulsatile GnRH stimulation is required, but the continuous exposure of the pituitary to GnRH causes desensitisation and a suppression of gonadotropin secretion. Due to Changes in the pulsatility of GnRH alter the ratio of secretion of the two pituitary gonadotropins throughout the menstrual cycle.[7]Excessive androgen in PCOS is related with increase in abdominal fat leads to dyslipidemia and hyperinsulinemia. Thus, hyperinsulinemia reduces hepatic sex hormone-binding globulin(SHBG) to increase circulating bioactive testosterone levels.[8]

Screening Methods of PCOS

Androgen Induced PCOS Model[9]:

Hyperandrogenism is the most common symptom of PCOS. One of the etiologic hypotheses for PCOS is that early life exposure to excessive androgens leads to PCOS later in life. Increased levels of circulating androgens in the rodent affected ovarian follicular maturation and cyst development, according to a study published more than 30 years ago. Several androgens, including dehydroepiandrosterone (DHEA), testosterone propionate (TP), and 5a-dihydrotestoterone, have been used to induce an acute PCOS condition in rats through regular injection or subcutaneous implants (DHT). However, there is still some inconsistency in the reporting of endocrine hormones and ovarian histology in different models. Furthermore, some studies did not look at cardiometabolic parameters or the effects of daily androgen injections and/or treatment on physiologic indices like body weight, stress indicators, or food intake. The pathological induction of PCOS in these rodent models is transient and dependent on androgen treatment. As a result, natural reproductive/ovarian cycling happens again after androgen administration is stopped.

DHEA Induced PCOS[9]:

The first androgen to increase in the female peripubertal cycle is dehydroepiandrosterone. Nearly half of follicular synthesised T can be obtained from circulating DHEA, and 25% of PCOS patients have higher-than-normal circulating DHEA levels. Roy et al. were the first to use dehydroepiandrosterone to induce PCOS in rats. DHEA (6 mg/100 g body weight, dissolved in 0.2 mL sesame oil) is injected daily for up to 20–27 days into prepubertal rats, typically aged 22 days. Rats become acyclic and anovulatory after treatment

Ovarian Morphology: Multiple follicular cysts varying in size from 0.45 to 2.2 mm in diameter, as well as degeneration of granulosa cell layers, grow in dehydroepiandrosterone-induced rats. The ovarian tunica capsule is not thickened, and the ovarian weight of DHEA-treated rats is substantially increased.

***Endocrine hormone profile*:** DHEA-induced rats have significantly higher serum DHEA, T, E2, FSH, LH, and PRL concentrations than control rats, while no changes in plasma FSH and LH concentrations have been identified by other groups. Fasting serum glucose and insulin concentrations were higher in DHEA-induced rats, indicating cardiometabolic abnormalities.

Early DHEA-related hyperandrogenemia, anovulation, cystic ovaries, and the production of insulin glucose metabolism abnormalities can all be detected using the DHEA-induced model.

TP- *Summary*: Induced PCOS Model[10]:

Testosterone propionate (TP) can cause hyperandrogenemia in rats when given prenatally or postnatally. Furthermore, prenatal T exposure during the crucial time of foetal development has been linked to reproductive system developmental and morphological abnormalities. Pregnant rats were given a single dose injection of T on gestational day 20 or T propionate (TP) from days 16 to 19 (3 mg T daily) of pregnancy for prenatal administration. Rats were given TP at a dose of 1.25 mg/100 g body weight at 5 days of age, or daily injections of 1 mg/100 g body weight from 21 to 56 days of age.

Estrous cyclicity: T prenatally treated rats had longer and more irregular estrous periods. Estrous cyclicity was disrupted and diestrus phase was persistent in postnatally treated rats**.**

Ovarian morphology: In the ovaries of rats treated prenatally with T, the number of preantral and antral follicles increased, whereas the number of pre-ovulatory follicles and corpus luteum (CL) cells decreased, as opposed to control rats. In prenatal T-treated rats, cystic follicles were also discovered. Rats given T postnatally, on the other hand, had massive cystic or atretic follicles and luteinization of theca cells in the ovaries. When postnatal T treated rats were fed a high fat diet, their body weight increased while their fasting glucose levels remained unchanged.

Summary: The ovary of rats treated with postnatal T showed morphological changes that mirrored the human PCOS phenotype. Prenatal T therapy, on the other hand, increased the number of preantral and antral follicles in rats, despite the fact that cystic follicles and ovary weight were unchanged in this model, and the reported changes did not match the ovarian morphology of people with PCOS. Both prenatal and postnatal T therapy increased serum T levels. Prenatal T administration had no effect on serum E2 and P4 levels, while continuous postnatal T treatment increased E2 levels, likely due to T conversion. An increased number of kisspeptin-positive cells in the ARC of

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prenatal T-treated ewes may be linked to defects in GnRH/LH secretion feedback control 11; however, one drawback of this study is that LH levels and ovarian morphology were not examined..

DHT induced PCOS models[10]:

Since DHT is not converted to E2 by aromatase, the PCOS phenotype in DHT-treated animals can be studied without taking into account the effects of oestrogen derived from androgens.

Prenatal DHT treated models:

Mice were injected with 250 g of DHT on days 16, 17, and 18 of gestation 28 to generate prenatal DHT-treated animals, while rats were given 3 mg of DHT daily from gestational day 16 to 19. The offspring were used as PCOS models that had been prenatally treated with DHT.

Estrous cyclicity: Prenatally administered DHT caused irregular cycles in rats and mice. The mice spent more days in diestrus and fewer days in proestrus than controls, resulting in fewer litters being produced every three months.

Ovarian morphology: Prenatal DHT treatment resulted in fewer normal large, antral, preovulatory follicles and CLs, as well as more atretic cyst like follicles. In prenatal DHT treated mice, CL and antral follicle wall areas were reduced, but the number of atretic cyst like follicles and the thickness of the antral follicle theca cell layer increased.

Neuropeptides in the hypothalamus: The number of kisspeptin and NKB positive cells in the ARC of the hypothalamus increased significantly in prenatal DHT treated rats, whereas the number of kisspeptin positive cells in the AVPV did not differ from that of control animals in diestrus. The input of aminobutyric acid (GABA) to GnRH-expressing neurons was increased in mice given DHT prenatally, according to a recent study.

Metabolic features and adiposity: Prenatal DHT-treated rats and mice had body weights that were close to control animals. Prenatal DHT therapy, on the other hand, increased adipocyte region in parametrial fat and the degree compared to the control group.

DHT prenatally treated rats and mice had abnormal estrous cycles and ovarian morphology similar to PCO. In prenatal ***Summary:*** DHT-treated rodents, increased LH levels were observed, along with an up regulation of kisspeptin in the ARC. There was no discernible difference in body weight, on the other side. This phenotype is similar to PCOS, which is marked by normal body weight and increased LH secretion.

Letrozole-Induced (Aromatase Inhibitor) Rodent Model of PCOS[11] :

Abnormal follicular development and polycystic ovary may result from intraovarian androgen excess caused by circulating hyperandrogenemia or abnormal steroidogenesis. P450 aromatase, which was expressed in the placenta, ovary, and testis as well as a wide variety of human tissues, converted testosterone and androstenedione into estradiol and estrone, respectively; a decrease in the enzyme's activity could result in increased ovarian androgen production and the development of PCOS. Aromatase is the key enzyme that converts T and androstenedione into E2 and estrone, respectively. It is widely expressed in human tissues, such as placenta, ovary, and testis. Reduced aromatase activity in the ovary is one of the pathophysiologic hypotheses of PCOS development. Letrozole is a nonsteroidal aromatase inhibitor that reduces conversion of androgens to estrogens in the ovary, resulting in increased T and decreased E2 production. Excess T in the ovaries is likely to cause polycystic ovaries directly in Letrozole-treated rats. The reduction in estrogen weakens the negative feedback on LH production in the pituitary, resulting in increased LH levels, which further stimulates theca cells to secrete T. Typically, 6-week-old female rats (puberty) are administered Letrozole orally at doses of 0.1, 0.5, and 1.0 mg/kg daily for 21 days, after which they become acyclic, with histological and biochemical features of human PCOS.

Estrous cyclicity: Regular vaginal smear examinations were used to monitor estrus cycles. In the analysis, only animals with two consecutive standard 4-day periods were used. The rats and mice treated with letrozole were fully acyclic. This rat model's vaginal smears showed an excess of leukocytes, the diestrus phase's predominant cell type.

Ovarian morphology: Ovaries from control group exhibited follicles in various stages of development including secondary follicles, graffian follicles, and fresh corpora lutea. In study groups, letrozole inhibited growth of follicles in a dose-dependent manner. Small follicles could be observed in early development, in addition to follicles showing evidence of atresia, and many large cysts with virtually no granulosa cell layer or large cystic follicles with scant granulosa cells. Ovaries from the sample groups had a higher rate of subcapsular ovarian cysts and capsular thickening than the control group. together with incomplete luteinization and a dose-dependent decrease in the amount of corpora lutea In some of the research classes, there was also evidence of theca cell hyperplasia..

Summary: Acyclicity, cystic ovarian morphology, elevated serum LH levels, and higher Kiss1 mRNA expression in the posterior hypothalamus are all observed in letrozole-induced PCOS model rats compared to control rats. This model accurately reproduces the metabolic characteristics of human PCOS, including a PCO-like morphology and elevated serum LH levels, and is thus suitable for studying human PCOS. Increased KNDy neuron activity was linked to a reduction in the negative feedback effect of sex steroid hormones, as evidenced by increased Kiss1 mRNA and serum LH levels.

Medicinal plants of natural origin-

Curcuma Longa (Turmeric) [12]**:**Curcumin is a water-insoluble polyphenolic curcuminoid derivative found in the rhizomes of Curcuma longa, an Indian spice (turmeric). Turmeric is widely used in Asian cuisine as a food additive

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and colouring agent, as well as in Indian herbal medicine. Curcumin makes up around 2–8% of all turmeric preparations. Curcumin has been shown to have a wide range of biological effects like Anti-inflammatory, antioxidant, hypoglycaemic, and antihyperlipidemic properties The study used virgin, cyclic, adult female Wistar Albino rats weighing 160–200 g. Once everyday for 21 days, followed by treatment with curcumin. Letrozole treatment resulted in abnormalities in the serum sex steroid profile, lipid profile, glucose, and glycosylated haemoglobin levels and antioxidant activity has been depleted. Whereas Curcumin was able to exert its calming effect by returning all parameters to normal and causing cysts in the ovaries to vanish. Curcumin, like Clomiphene citrate, has a number of beneficial effects in the treatment of PCOS.

Aloe barbadensis (Aloe)[13]: Aloe barbadensis Mill. (Liliaceae) is a well-known plant with such properties. Polyphenols, sterols, flavanoids, and other nutrients were analysed qualitatively and quantitatively for polyphenols, sterols, flavanoids, and other nutrients in the Aloe vera gel formulation. To induce PCOS, five-month-old Charles Foster female rats were orally fed letrozole, a non-steroidal aromatase inhibitor. The rats were then given the Aloe vera gel formulation orally.AVG treatment of PCO rats resulted in a reduction in ovary atretic cysts as compared to PCOS controls, according to histological review. By restoring ovarian steroid status and modifying main steroidogenic behaviour, aloe vera gel formulation protects against the PCOS phenotype.

Glycyrrhiza glabra (liquorice)[14]: Traditional medicine has used liquorice (Glycyrrhiza glabra of the Leguminosae family) to treat a variety of ailments. Antifungal, antiviral, antibacterial, and antihyperglycemic properties are all present in it. The most bioactive compound in liquorice is glycyrrhizic acid. Phytoestrogens found in liquorice include liquiritigenin, liquiritin, isoliquiritin, isoliquiritigenin, glabidin, and glabrene. The effects of two natural compounds derived from liquorice root on vascular tissues in vitro and in vivo were reported: glabridin, the main glabrene, and isoflavane, an isoflavene, both demonstrated estrogen-like activities. One of the bioactive compounds responsible for weight loss may be liquiritigenin, a selective oestrogen receptor ligand. Some molecules, such as glabrene and glabridin, have been shown to reduce weight in vivo. It has also been documented that treating hirsute women with a combination of spironolactone and liquorice may help with PCOS by reducing the volume depletion caused by spiranolactone and possibly increasing its anti-androgenic activity.

Mentha piperita(Peppermint) [15]: Peppermint (Mentha piperita L.) is a member of the Labiatae family that originated in the Mediterranean region and is now widely cultivated all over the world. Antioxidant, antitumor, antiallergenic, anti-inflammatory, antiviral, antibacterial, and antifungal properties are all present in peppermint. It also has anti-androgenic properties, lowering the level of free testosterone in the blood after three weeks of treatment with letrozole and peppermint. Females with PCOS had significant changes in serum testosterone, oestrogen, LH, and FSH function. Ovarian cysts with a reduced granulosa layer, atretic follicles, and a small number of corpora lutea were found in the PCOS community. Peppermint was found to have a strong potential as an alternative therapy in the treatment of PCOS, as shown by necrosis in stromal mesenchymal cells, hyperplasia of luminal epithelial cells, and necrosis in stromal mesenchymal cells.

Allium fistulosum (Onion)[16]: In Asian countries, the Welsh onion (Allium fistulosum) is well-known for its use in food and traditional medicine. For treatment, administered AF extract to letrozole-treated rats for 2 weeks. In terms of serum hormonal levels, the LH/FSH ratio and serum oestrogen levels were positively affected by AF extract therapy. FSH and LH are necessary for ovulation, and PCOS patients often have a two- to three-fold increased LH/FSH ratio, which is enough to cause ovulation disruption. The findings suggest that AF extract normalises follicular growth and ovarian cysts. In the letrozole-induced PCOS rat model, the steroid hormone-related receptors demonstrated restoration of m-RNA expression after treatment with AF extract A. fistulosum extract treatment relieved hormonal imbalance and altered ovarian function.

Linum usittassimum (Flaxseed)[17]: Flaxseed is made from Linum usittassimum (Linaceae), an omega-3 fatty acid-rich food that is also one of the best sources of dietary lignin. ALA, lignans (secoisolariciresinol diglycoside-SDG), and soluble flaxseed fibre mucilage (d-Xylose, L-Galactose, L-Rhamnose, d-galacturonic acid) are all biologically active compounds with major health benefits. Flaxseed or isolated lignan has been shown in studies to lower androgen levels while also normalising lipid levels. Lignans seem to minimise excess testosterone, which is a crucial factor in the development of PCOS. Flaxseed supplementation can help women with PCOS control androgen levels, according to a case study. The study found a substantial reduction in androgen levels. There was also a decrease in hirsutism. Flaxseed can have a significant effect on testosterone levels, as well as symptoms associated with hyperandrogenism, such as hirsutism, according to the findings. Another research looked at the impact of flax seeds on ovarian morphology in PCOS patients, finding that flax seed supplementation decreased ovarian volume, increased the amount of follicles in the ovaries, and improved menstrual cycle duration. However, hirsutism, blood sugar levels, or body weight did not improve as a result of the research.

Panax ginseng (Ginseng)[18]: Herbal medication is made from the roots of Panax ginseng (Araliaceae). It has anti-aging properties and is used as a tonic. Ginseng saponins are ginseng's active ingredient. Rb1, Rb2, Rc, Rd, Re, Ro, Ra, and minor ginsenosides make up these ginsenosides. It can be used as a dietary supplement. Estradiol valerate induced polycystic ovary in rats. The ovarian morphology was examined in this analysis. The ginseng-containing formulation is known as Kampo preparations. It is formulation significantly decreases the plasma LH levels

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and thereby it is effective in improving endocrine condition in the treatment of disturbances of ovulation in patients with PCOS.

Tribulus terrestris (Puncture vine)[19]: Puncture vine or Devil's eyelashes, Tribulus terrestris (Zygophyllaceae), plays an important role in traditional medicine. The herb Tribulus terrestris has been shown to help with polycystic ovarian syndrome. Tribulus terrestris extract was found to be successful in improving ovulation in rats with polycystic ovaries induced with estradiol valerate in a study. The extract treatment improved ovarian follicular development and normalised estrous cyclicity and steroidal hormone levels. Many herbalists believe that tribulus is an excellent overall ovarian stimulant and female fertility tonic for women with polycystic ovary syndrome.

Gymnema sylvestre (Gymnema)[20]: Gymnema sylvestre (Asclepiadaceae) is an Ayurvedic herb that has been used for thousands of years. It has a wide range of pharmacological effects, including anti-diabetic, hypoglycemic, and lipid-lowering properties. Saponins, especially gymnemic acids, are the active constituents in gymnema. Gymnema has been shown to have hypoglycemic properties in diabetic animal models. It keeps blood glucose levels in check. Metformin therapy is a convenient way to treat PCOS. Gymnema can thus be used to treat the root cause of insulin resistance. Gymnema is a good choice for PCOS because of its insulin-modulating properties and the added advantage of lowering the high triglycerides that come with the condition.

Punica granatum (Pomegranate)[21]: Pomegranate (Punica granatum of the Punicaceae family) is a fruit with a wide range of medicinal properties. Folic acid, vitamins (B2, C, B1), carbohydrates, pantothenic acid, and organic acids are all contained in the fruit. Unsaturated and saturated fatty acids are said to be present in the crop. In adult female rats, the effect of pomegranate extract in the control or management of PCOS was studied using a control and a PCOS community. The levels of free testosterone, serum oestrogen, and andrestandoin hormone were measured in the experimental community. Pomegranate extract seems to have a protective impact on polycystic ovarian syndrome hormonal imbalances, according to the report. The extract's phenolic compounds and phytosterols have been shown to help alleviate PCOS complications. Consumption of the extract, according to the report, decreases the complications associated with PCOS.

Symplocos racemosa (Lodh Tree)[22]: Symplocos racemosa Roxb, a member of the Symplocaceae family, is a common Ayurvedic remedy for female problems. It's also known as Lodhra, and it's used as a single medication or in multi-component formulations and preparations in Indian medicine. In a Letrozole-induced female rat model, the anti-androgenic properties of S. racemosa were investigated in the treatment of PCOS. Treatment with Symplocos racemosa resulted in substantial improvements in oestrogen, testosterone, progesterone, and ovarian tissue levels. It improves fertility and prevents ovarian cell dysfunction in PCOS patients.

Cinnamomum zeylanicum (Cinnamon)[23]: Cinnamon (Cinnamomum zeylanicum of the Lauraceae family) is an insulin potentiator. Insulin-stimulated glucose uptake and glycogen synthesis are controlled by this compound. Fasting and oral glucose tolerance test values were assessed in fifteen women with PCOS in a pilot study. In women with PCOS, the cinnamon extract increased insulin sensitivity. Cinnamon extract contains polyphenols and procyanidins, which potentiate the insulin signalling pathway, resulting in a hypoglycemic impact. Cinnamon's function as an adjunctive therapy in the treatment of PCOS was identified in this research. Cinnamon's impact on menstrual cyclicity and metabolic dysfunction in women with PCOS was studied in another research. It was a 45-woman randomised controlled trial. Oral cinnamon supplements were given. Menstrual cyclicity, luteal phase, and progesterone levels were all tracked. Cinnamon supplementation increased menstrual cyclicity and was shown to be beneficial in the treatment of polycystic ovary syndrome.

Vitex Negundo(Chaste Tree)[24]:Vitex negundo is a plant belonging to the (Linn) Verbenaceae family, genus Vitex, and species negundo. It's the five-leaved chaste flower, also known as monk's pepper. It has been documented to have anti-inflammatory, analgesic, antioxidant, antifungal, antiviral, and anti-inflammatory properties, as well as being used in gynaecological disorders. It also has anti-androgenic and estrogenic properties (linoleic acid-like estrogenic compounds). For the induction of PCOS, letrozole was given orally (p.o) for a duration of 21 days. The rats were then given extract of vitex negundo, which has positive effects on the ovary as well as effects on glucose tolerance, estrous cycle irregularities, LH: FSH ratio, steroidogenic enzymes, and cardiovascular parameters. It was able to successfully treat the rats with extract, which caused abnormalities in serum sex steroid profile, lipid profile, glucose, and estrous cycle. This may be attributed to the extract's phyto-components.

CONCLUSION:

The most common cause of menstrual irregularities and hyperandrogenism is polycystic ovary syndrome (PCOS). It is the most common cause of female infertility. Several risk factors for PCOS have been studied, including glucose intolerances, obesity, and dyslipidemia. Many treatments are currently available, but they are associated with moderate to serious side effects, and their high cost has led to a search for plant-based remedies to treat PCOS. In this study, summarize some of the most important medicinal plants for treating PCOS and helps with PCOD symptom relief and management. Hyperandrogenism, insulin sensitivity, fertility, and menstrual cyclicity are all aided by these plants.

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SDC-PC BASED SOLID SEDDS OF BCS CLASS II DRUG

Aneri V. Adsul[1]*, Bharatee P. Chaudhari[2] and Vivekkumar K. Redasani[3]

[1]Student, Department of Pharmaceutics, YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Satara. Maharashtra, India.

[2]Assistant Professor, Department of Pharmaceutics, YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Satara. Maharashtra, India.

[3]Principal, Department of Pharmaceutics, YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Satara. Maharashtra, India.

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***Corresponding Author**
Aneri V. Adsul
Student, Department of Pharmaceutics, YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Satara. Maharashtra, India.
aneri14597@gmail.com

ABSTRACT

Aim: The purpose of this research is to develop, optimise, and analyse losartan potassium loaded solid SEDDS using a newly synthesised surfactant. Losartan potassium is an angiotensin II receptor blocker that belongs to the BCS class III of antihypertensive drugs. Solid SEDDS were used to increase the permeability, oral bioavailability, and first pass metabolism of losartan potassium, which had a low permeability and oral bioavailability. **Material and methods:** As a surfactant, SDC-PC was synthesized. The solubility of the API in various oils, surfactants, and co-surfactants was investigated, and oleic was chosen as the oil phase, SDC-PC as the surfactant, and PEG 400 as a co-surfactant for formulation. A pseudo-ternary phase diagram was created to get the ideal emulsification area. SEDDS liquid was prepared and tested. Following an evaluation, it was discovered that LP4 was stable and optimum. **Result and discussion:** Aerosil 200 was used as a carrier to convert the formulation into solid SEDDS. The formulations were compared to LOSAR®, as marketed product. On in-vitro drug release, optimised batch LP4 was shown to have similar drug release to the marketed formulation.

KEYWORDS: Losartan potassium, SEDDS, Surfactant, Solid dosage form, Pseudo-ternary phase diagram.

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INTRODUCTION

Losartan potassium (LP) is a non-peptide angiotensin II receptor antagonist (Type AT1) that is orally active and undergoes substantial first-pass metabolism by the cytochrome P450 enzyme, with 14 % of the dose converting to an active metabolite.[1,2] LP is a class III drug that comes in the form of a white to off-white free-flowing crystalline powder with a log P value of 5.37, a half-life of about 2 hours, and a systemic bioavailability of about 33%.[1,2,3] The drug is used once or twice a day as a 25 mg tablet, with total daily doses ranging from 25 to 100 mg.[2] In diabetic patients, LP provides beneficial pressure control, lowering the risk of stroke and the progression of renal disease to the terminal stage.[4] LP binds to plasma proteins extensively and can cause gastrointestinal problems, neutropenia, active hepatotoxicity, migraines, and pancreatitis.[5] To reduce the frequency of dose and adverse effects of LP, sustained drug delivery is required to prolong the drug release, and self-emulsifying drug delivery can be employed to achieve this.[3]

Self emulsifying formulations are defined as isotropic mixtures of natural or synthetic oil, liquid or solid surfactant or one or more hydrophilic solvents and co-solvent or surfactants.[6] Upon mild agitation followed by dilution in aq-media, such as GI fluids, these system can form oil-in-water(o/w) emulsion(10-100nm)[7]. SEDDS' physical qualities, as well as the chemical structures of its constituents, were found to be important determinants of application and tolerability.[8] These fine microemulsion droplets have the benefit of providing the drug in a dissolved form with a large interfacial surface area for drug absorption, resulting in improved, uniform, and repeatable bioavailability.[9] The oral bioavailability of both hydrophobic and hydrophilic drugs can be improved by increasing membrane fluidity to facilitate transcellular absorption, opening tight junctions to allow paracellular transport, inhibiting cytochrome P450 as isoenzyme in the intestinal region, and inhibiting efflux pumps such as P-glycoprotein.[10] Using a self-emulsifying drug delivery system to improve oral bioavailability of BCS class III medicines has a ton of potential. These systems have been found to be useful in the formulation of first-pass metabolism medications as well as orally delivered pharmaceuticals that obtain access to the systemic circulation through direct absorption into the intestinal lymphatic system.[11]

Following mechanisms are implicated for the improvement of permeability.

- Gastric retention time – The oil in SEDDS can decrease the gastric empting time.

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- Lymphatic transport – The oil in SEDDS may enhance the lymphatic transport and the bioavailability of highly lipophilic drugs by promoting their association with chylomicrons in the enterocytes and avoiding hepatic metabolic pathway.
- Intestinal protein efflux – oil and non-ionic surfactants in SEDDS may reversibly inhibit P-glycoprotein and the multidrug resistance related proteins -2 efflux transport or increases the transcellular permeability.
- First pass metabolism – SEDDS may inhibit the action of cytochrome P450 enzyme which metabolizes drug in intestinal wall.[12]

Because liquid SEDDS have disadvantages including instability, low convenience, production processes, interaction during filling in capsule shells, and storage temperature, solid SEDDS were developed. When compared to precursor liquid SEDDS, solidification provides a number of advantages, which can be summarised as better drug solubility and dissolution, improved safety, controlled or sustained drug release, and industrial and commercial benefits.[13]

The aim of our present study to develop, optimize and evaluate losartan potassium loaded solid SEDDS using synthesized surfactant. As surfactant plays important role to reduce surface tension between two different phases. The vesicular based system act as reservoir for the control release of a number of active drug including antibiotics, corticosteroids. They also act as permeation enhancer in systemic absorption.[14]

MATERIALS AND METHODS

Losartan potassium was obtained from YARROW CHEM PRODUCTS, Mumbai. Oleic acid was obtained from S. D. Lab chemical centre, Mumbai. Aerosil 200 was obtained from Gangwal chemical, Mumbai. Stearoyl chloride was obtained from Dolphin pharmacy instrument Pvt. LTD, Mumbai. Sulfanilamide, acetone, n-hexane, ethyl acetate, caster oil, iso propyl myristate, PEG 400, Tween 80. all the chemicals and solvents used in this work belonged to analytical grade.

Synthesis of surfactat

N-((4-sulfamoylphenyl) carbamothioyl) stearamide (SDC-PC) was synthesized by taking stearoyl chloride 0.67 mL (2 mmol) and KSCN 194 mg (2 mmol) in 50 mL round bottom flask equipped with reflux condenser in 20 mL acetone. The resulting mixture was then stirred for 2 hours at 60 °C. After 2 hours, Sulfanilamide 344 mg (2 mmol) was added and

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refluxed the reaction mixture for further 18 hours (Scheme 1). The progress of the reaction was monitored periodically using TLC in ethyl acetate and n-hexane (3:7, v/v) solvent system.[14]

Evaluation of synthesized surfactant

% Practical yield

Percent practical yield was calculated by following formula:

% practical yield = $\text{Practical yield} \div \text{Therotical yield} \times 100$

Melting point

Melting point apparatus was used to determine the melting point of SDC-PC. In the melting point apparatus, a little amount of SDC-PC was inserted in one end of a closed capillary and the temperature at which the drug melted was recorded and compared to previous research.

ATR-FTIR Spectroscopy

The ATR-FTIR spectrum of surfactant was collected using the ATR-FTIR instrument BRUKER-Alpha 100508. A small amount of drug was collected and applied directly to the ATR diamond. A pressure pump was used to push the medication. The spectrum was obtained by combining 24 scans across a range of 4000-400cm.[15] The precise wavelength of light was partially absorbed by the sample, and at least one was reflected off the internal surface in contact with the sample. The ATR-FTIR spectrum depicts percent transmittance in terms of light wavelength (cm). For all interaction, the spectrum of the sample was acquired and compared to the spectrum of the pure drug.[16]

Critical Micelles Concentration (CMC) Determination

The CMC of all the newly synthesized surfactants were determined spectrophotometrically using UV-visible spectrophotometer (Shimadzu, UV-1800, Japan). Surfactant were dissolved in ethanol in different concentrations i.e. 0.01–0.1 mM read spectrophotometrically. A plot for each concentration versus its absorption was made and then straight lines were drawn on the values. The critical micelle concentration was the point where two straight lines intersect each other on this graph of concentration vs absorption.[14]

Solubility study

Solubility studies in various oils, surfactants, and co-solvents were conducted in order to determine the optimal SEDDS excipients with good solubilizing capacity for losartan

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potassium.[17] In a glass vail containing an excess of Losartan potassium, one (1) ml of each of the selected oil, surfactant, and co-surfactant sample was added (50-70 mg). To achieve equilibrium, the vails were shaken for 72 hours on an orbital shaker at 40 ° C.. After that, aliquots of the supernatants were taken and filtered using a 0.45m membrane filter. Filtration with a 0.45m membrane filter separated the unmixed drug. The filtered sample was centrifuged for 15 minutes at 3000 rpm.

Construction of ternary phase diagram

Phase diagram provide useful platform for delineating the area of microemulsion. Ternary phase diagrams of oil, surfactant/co-surfactant (Smix) and water were developed using the water titration method.[19] Surfactant and co-surfactant were mixed up with six different (Km) weight ratios 1:9 to 9:1. For each phase diagram oil to specific Smix ratio was mixed in different proportion from 0.5:4.5 to 4.5:0.5. Nine different proportions are 0.5:4.5, 4:1, 3.5:1.5, 3:2, 2.5:2.5, 2:3, 1.5:3.5, 4:1 and 4.5:0.5. This made to maximum ratios were covered for the study to delineate the boundaries of phase precisely formed in the phase diagram. A transparent and homogenous mixture of oil/Smix was formed by using magnetic stirring. Then each mixture was titrated with drop wise addition under gentle agitation until the required clarity and flow ability was achieved. The point at which system become turbid, these points were recorded. Corresponding to these points calculate the % w/w combination of oil, surfactant and co-surfactant. Using these points phase diagram was constructed to determine the boundaries of microemulsion reason.[20] The phase diagram constructed using CHEMIX school software version 7.0.

Preparation of liquid sedds

By dissolving the amount of Losartan potassium as shown in the ternary phase diagram, a number of SEDDS formulations were created. The oil phase (125 mg) was placed in a vial, and the drug (25 mg of LP) was added straight to this vial and combined using a vortex mixer. To make an isotropic mixture, a sufficient amount of Smix was added to the oil-drug mixture and vortexed, followed by homogenization for 10 minutes. The formulation was checked for turbidity and phase separation before being stored at room temperature until further use.[21]

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Evaluation of liquid sedds

Thermodynamic stability study

Heating cooling cycle (Freeze-thaw cycle): Six cycles of heating at 45˚C (incubator) and cooling at 4˚C (refrigerator) was conducted for not less than 48hrs at each temperature.

Centrifugation test: Those formulations which passed the heating cooling cycle test then subjected to centrifugation test at 3500 rpm for 30 min. Those formulations that did not show any sign of phase separations, which are most thermodynamically stable.[22]

Dispersibility test

The efficiency of self-micro emulsifying drug delivery system was evaluated by the dispersibility test. Dipersibility study was performed by adding each formulation in 500 ml of distilled water at 37˚C ± 0.5˚C. A standard stainless steel dissolution paddle rotating at 50 rpm provided gentle agitation. The in vitro performance of the formulation was visually evaluated using the following grading system.

- **Grade A:** Rapidly forming emulsion having a clear or bluish appearance (within 1 min)
- **Grade B:** Rapidly forming slightly less clear emulsion, having a bluish white appearance.
- **Grade C:** Fine milky emulsion was formed (Within 2 min).
- **Grade D:** Dull, grayish white emulsion having slightly oily appearance (Longer than 2 min).
- **Grade E:** Formula exhibiting either poor or minimal emulsification with large oil globules present on the surface.[16]

Self emulsification time

A standard USP dissolution apparatus type II was used to test the self-emulsification efficiency of SEDDS. In 900 ml of 0.1N HCI kept at 37 °C, a quantity equivalent to 25mg of each formula's microemulsion was added. A typical stainless steel dissolving paddle moving at 75 rpm provides agitation. The rate of emulsification and final appearance of the microemulsion were visually analysed for the created formulae. These investigations were carried out in order to better replicate the state of the stomach following oral ingestion. With respect to time, the tendency to emulsify spontaneously and the progression of emulsion droplets were observed.[23]

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Globules size measurement and PDI

In a beaker, SEDDS formulation (1 ml) was diluted with 100 ml deionized water and constantly stirred with a glass rod. The microemulsion that resulted was then tested for globule size and PDI. Dynamic light scattering with particle size apparatus was used to determine the globule size and PDI of the resulting micrommalsion (Malvern Zetasizer, Ver. 7.12, serial Number: MAL 1098084, UK). The particle (droplet) size was measured at equilibrium. The lowest droplet size values that are more stable, isotropic, and transparent oil/water (o/w) dispersions that have a higher absorption rate potential.[24]

Zeta potential

Zeta potential is the electric potential in the interfacial double layer. Zeta potential is a key indicator of stability. It is indicating the electrostatic repulsion and congregation in oily droplets. The electrostatic repulsion of emulsion droplets plays an important role for assessment of stability of the system High electrostatic repulsion droplets prevent coagulation or flocculation on to fine emulsion droplets into larger oily globules.[25] Zeta potential determined by Zetasizer was monitored at 25°C at a scattering angle 173 (Malvern Zetasizer. Ver. 7.12, serial Number: MAL 1098084. UK)

% Transmittance

The percent clarity of the prepared samples was assessed to demonstrate the formulation's transparency. Using a UV-spectrophotometer and distilled water as a blank, the % transmittance of the system is determined at 650 nm wavelength. If the percent transmittance of a formulation is greater than 99 percent, the formulation is transparent.[21]

Drug content

The drug content was determined by dissolving SEDDS formulation equivalent to 10 mg drug in 50 ml of methanol and mixed well with shaking for two to three times 0.1 ml of this solution was diluted with fresh methanol, and drug content was determined spectrophotometrically (Shimadza 1800, Japan) at 233nm.[26]

Preparation of solid sedds

By using an adsorption approach, liquid SEDDS were turned into free-flowing powders, resulting in a more uniform drug release profile. Drops of liquid SEDDS were added to aerosil 200 in 1:0.25, 1:0.5, 1:1, 1:1.5, and 1:2 ratios, and the mixture was stirred for 5 minutes in a mortar pestle. To ensure a consistent dispersion of the formulation, the mixture

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was homogenised with a glass rod after each addition. The final mass was passed through mesh no. 120 (0.125mm), dried at room temperature, and stored in desiccators until further examination.[16,27]

Evaluation of solid sedds

Flow properties

Bulk density, tapped density, Hausner's ratio, Carr's index, angle of repose was evaluated.

Reconstitution properties of solid SEDDS

Effect of dilution on solid SEDDS

The property of quick emulsification was noticed after 100 mg S-SEDDS was correctly weighed and added to 100 ml distilled water in a beaker at 37°C and gently stirred with a magnetic stirrer at 100 rpm. The tendency to produce an emulsion was determined as follows:

- Good - If emulsification occurs in <1 min with clear or transparent emulsion.
- Bad – If emulsion if less clear or transparent[16]

In vitro dissolution test

The drug dissolution profile was studied using the USP dissolution apparatus II (Electrolab, Mumbai). Dissolution tests were performed in 900 ml of 0.01 N HCI (pH 2.0) at 37 0.5 °C with 75 rpm stirring. 10 mg of LP and formulation batches were introduced to the dissolution medium, and 5 ml samples were withdrawn after 10, 20, 30, 40, 50, and 60 minutes, and replaced with 5 ml fresh 0.5 percent Polysorbate 20 in 0.01 N HCL each time. The solutions were immediately filtered through a 0.45 um membrane filter, diluted, and UV-spectrophotometrically measured at 233nm.[28]

Drug and excipient interaction

ATR-FTIR spectra of pure LP, Aerosil 200, physical mixtires and Solid SEDDS formulations were recorded by ATR-FTIR Spectrometer (ALPHA 100508 BRUKER. US) to illustrate the promising interactions among the excipients used in the formulation. The spectrum was scanned over the wave number range of 4000-400 cm^{-1}[16]

RESULT AND DISCUSSION

% Practical yield

% Practical yield of synthesized surfactant was found to be 84.32%.

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Melting point

Melting point of synthesized surfactant was found to be 135˚C

Fourier transforms infrared (FTIR) spectroscopy

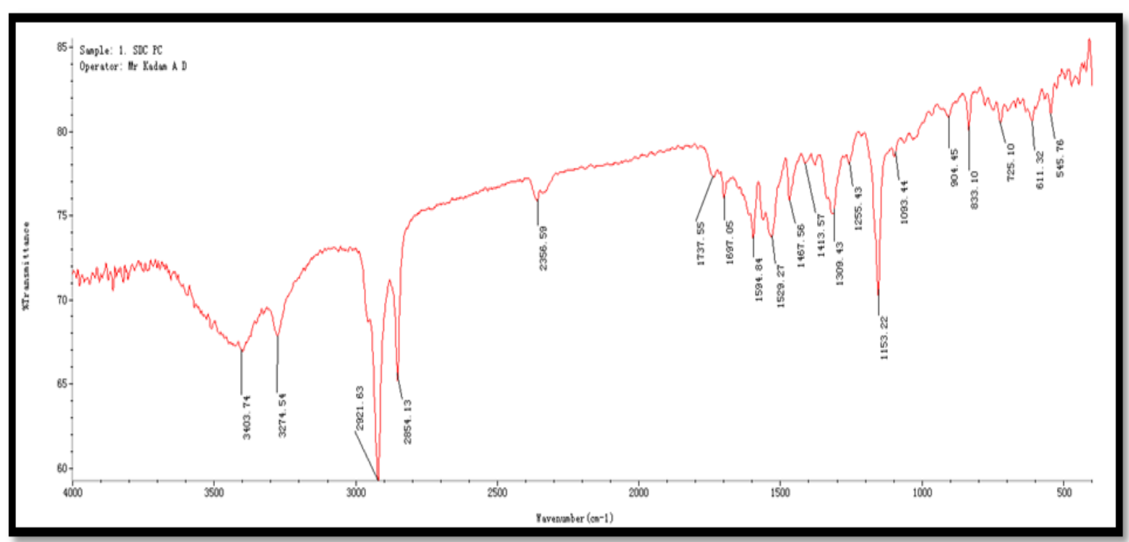

Fig. 1: ATR-FTIR spectrum of SDC-PC.

ATR-FRIR spectrum of SDC-PC is given in above figure. The spectrum shows NH_2 asymmetric at 3403.74 cm^{-1}, NH at 3274.54 cm^{-1}, CH_2 asymmetric at 2921.63 cm^{-1}, CH_2 symmetric at 2854.13 cm^{-1}, C=C aromatic at 1529.27 cm^{-1}, C-N at 1309.43 cm^{-1}, C-S at 1153.22 cm^{-1}. All characteristic peak of surfactant found in reported range.

Critical micelles concentration

The critical micelles concentration is an important phenomenon for scientist and researchers in the field of drug delivery. The micellization is the property of non ionic surfactant and polymers. No-ionic surfactant when exposed to the water forms aggregations called as micelles. When surfactants are added to a solvent, they are dispersed in the solution. By increasing the concentration of surfactant i the medium, at CMC they form micelles. CMC can be found by plotting graph of suitable physical property as a function of surfactant concentration. The CMC values of synthesized surfactant were determined by plotting the absorbance at λ_{max} against the concentration of each surfactant. The surfactant SDC-PC was read ranging from 0.01-0.1 µg/ml concentration and its CMC was calculated as 0.06mM.

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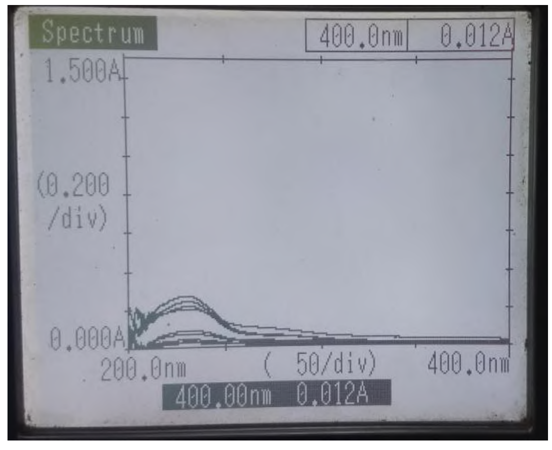

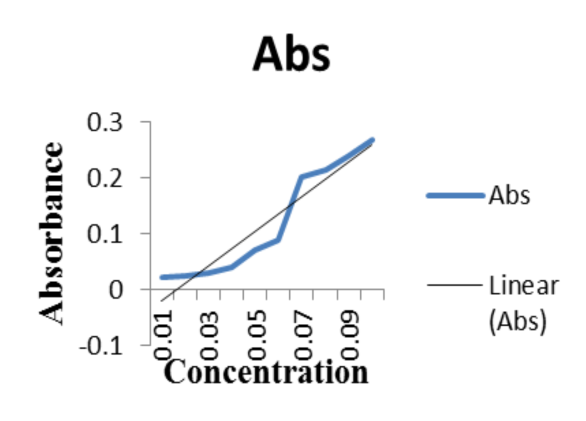

Fig 2: UV-visible spectra and CMC of SDC-PC.

Solubility study

After performing solubility study, the drug was found to be more soluble in oleic acid (oil), SDC-PC (surfactant), PEG400 (co-surfactant) results are shown in fig.

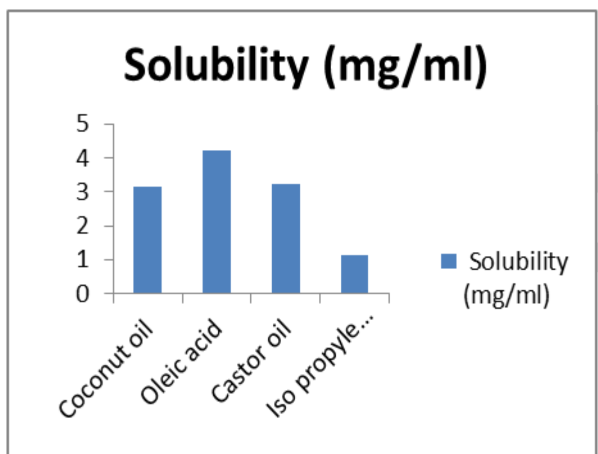

Fig. 3: solubility in various oils.

Fig. 4: Solubility in various surfactant.

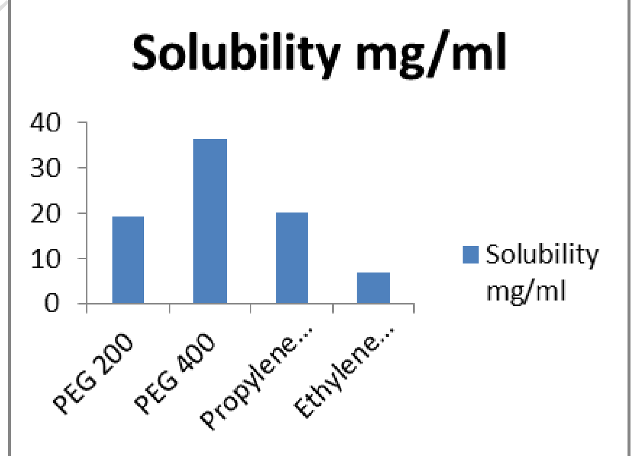

Fig. 5: Solubility in various co-surfactant.

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Construction of ternary phase diagram

The pseudo-ternary phase diagram is useful to determine the suitable combination of oil, surfactant and co-surfactant concentration in the formulation to form the nano-emulsion. After selection of oil, surfactant and co-surfactant based on the solubility study, the pseudo-ternary phase diagrams containing a fixed ratio of surfactant and co-surfactant (Smix) were constructed. The ternary phase diagram of the system containing SDC-PC: PEG 400 with the ratio of 1:9 formed with the wider emulsifying region in the presence of oleic acid as oil. It is clear from Fig.7 that the emulsifying region increases with an increase in the amount of surfactant mixture concentration in the system. Several reports explained this phenomenon of a decrease in the mean droplet size as an outcome of an increase in the concentration of surfactant and vice versa. The reduced droplet size with a high concentration of surfactant mechanism may be supported with the following statements:

- Stabilization of the oil droplet occurs with reduction in the interfacial tension between oil and water phase at a high concentration of surfactant.
- Enhancement of water penetration into oil in the presence of high surfactant which causes the release of oil droplets in aqueous phase

The addition of the drug did not affect the self-emulsifying region significantly.

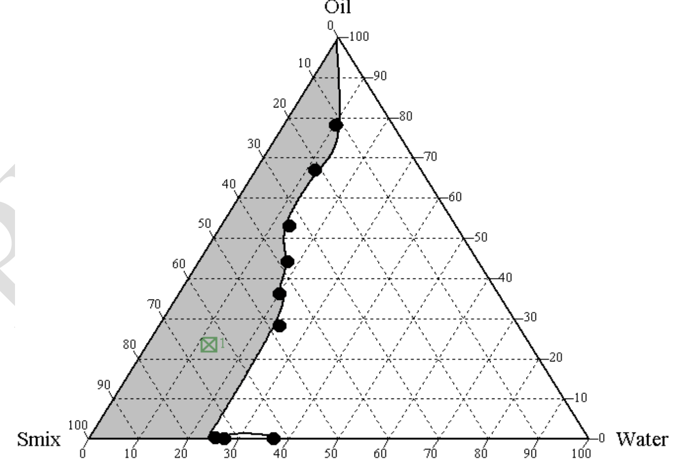

Fig. 7: Ternary phase diagram (Smix 1:9).

Preparation of liquid SEDDS

A total of four (4) formulations LP1 to LP4 were successfully prepared with their respective composition as shown in table 1.

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Table 1: Formulation table of Liquid SEDDS.

Batch code	Drug (mg)	Oil (%)	$Smix_1$ (%)	$Smix_2$ (%)	Weight of batch (%)
LP1	25	70	-	30	100
LP2	25	60	-	40	100
LP3	25	50	-	50	100
LP4	25	40	-	60	100

Thermodynamic stability study

The thermodynamic studies have always helped determine the kinetic stability of the formulation. The main criteria of microemulsion for pass this test is not to show any indication of phase separation, creaming, cracking or coalescence. All the prepared formulations had passed the thermodynamic study test, with no signs of phase separation and precipitation of drugs. This indicates that the prepared formulations were stable against the maintained storage conditions.

Dispersibility test

The dispersibility of microemulsion shows Grade A of all formulations showed a rapidly forming emulsion having a clear or bluish appearance (within 1 min).

Table 2: Dispersibility grades and self emulsification time.

Sr. No.	Batch	Dispersibility Grade	Self emulsification time (sec)
1	LP1	A	54
2	LP2	A	48
3	LP3	A	45
4	LP4	A	44

Self emusification time study

The emulsification time of all batches was found to be in the range of 44 to 54 seconds as shown in Table 2. The batch LP4 showed very short emulsification time. The determination of self emification time for the assesment of microemulsion spread or scatters in GIT medium. Smaller the size of particle faster the lease and shows the efficiency of formulation.

Globule size, PDI and Zeta potential

Globule size of emulsion plays important role in absorption and also in stability. Globule size of optimized formulation was observed 836.7 nm (Fig.8) which is within the range of nano-emulsion (500-1000nm) and polydispersity index (PDI) was observed 0.466. Zeta potential of

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optimized batch was observed -0.329 mV (Fig.9) negative potential around particles shows improved lymphatic uptake of system.

Table No.: Globule size, PDI and zeta potential of all batches.

Batch	Globule size	PDI	Zeta potential
LP1	583.8	0.975	0.527
LP2	1671	0.040	0.0608
LP3	974.6	0.604	-0.0518
LP4	836.7	0.466	-0.329

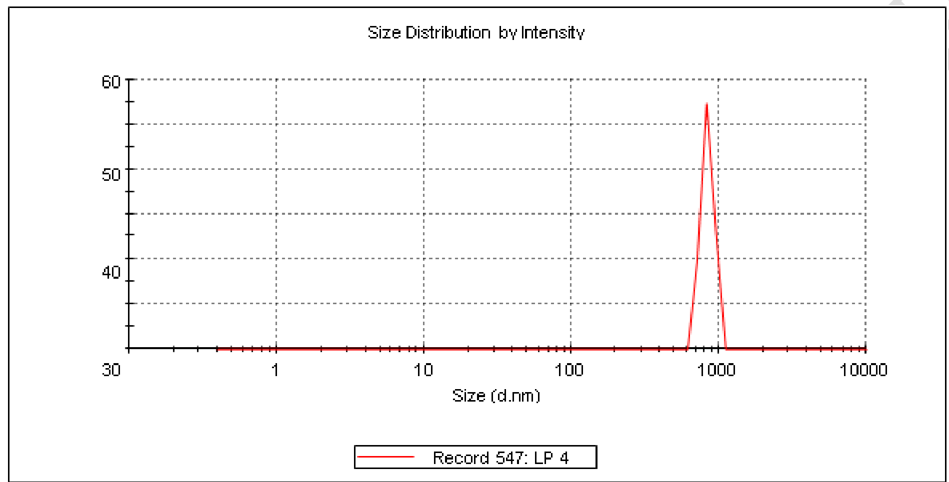

Fig. 8: Globule size distribution graph of optimized batch.

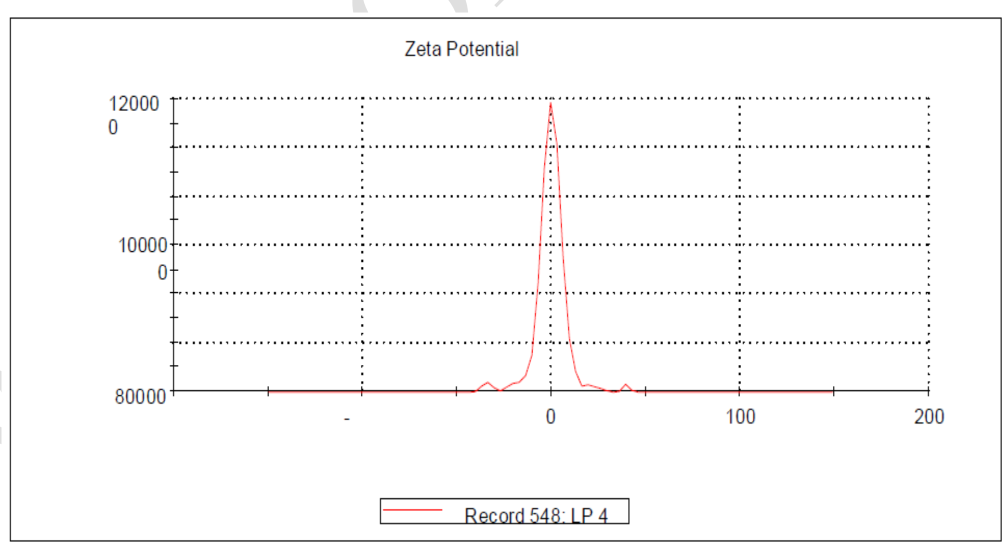

Fig. 9: Zeta potential of optimized batch.

% Transmittance measurement

Percent transmittance was evaluated for proving the transparency of formulation. A value closer to 100% confirms, the transparency of the formulation and indicates large surface area

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for drug release. Percent transmittance of formulation was found to be in the range of 100% to 93.2 % and tabulated in Table 3.

Table 3: % transmittance measurement.

Sr. No.	Batch	% Transmittance
1	LP1	96.1
2	LP2	98.3
3	LP3	99.0
4	LP4	100.3

Drug content

The drug content of the prepared losartan potassium loaded SEDDS was determined to evaluate the uniformity of dose in the formulation. The drug content in different prepared batches is listed in table 29. Drug content of liquid SEDDS formulation batch LP4 was found to be highest as 98.81%. So it was considered as obtimized batch for further evaluation.

Table 4: Drug content of liquid SEDDS formulation.

Sr. No.	Batch	Drug content (%)
1	LP1	98.32
2	LP2	97.56
3	LP3	98.79
4	LP4	98.81

Formulation of solid SEDDS

From the evaluation of liquid SEDDS it was observed that LP4 was optimized batch so LP1, LP2, LP3, LPV4 batches are converted to solid SEDDS to avoid stability problems. Solid SEDDS formulations were prepared by using the carrier i.e. Aerosil 200. Optimized liquid SEDDS converted into solid SEDDS by using adsorption technique. The solid carrier demonstrated to be effective to construct free flowing powder form of liquid SEDDS with high surface area. The amount of carrier required to absorb the liquid SEDDS was strongly associated with the surface area of adsorbant.

Table 6: Formulation batches of solid SEDDS.

Batch code	Liquid SEDDS (mg)	Aerosil 200 (mg)	Total wt. of tablet (mg)
LP1	350	250	600
LP2	350	250	600
LP3	350	250	600
LP4	350	250	600

*Calculation for one dose; batch size 20 tablets

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Evaluation of Solid self emulsifying drug delivery

Flow properties

Flow properties of solid SEDDS such as angle of repose, bulk density, tapped density, carr's index and hausner's ratio are deteremined and found that the prepared solid SEDDS showed "Good" flow properties as showed in Table 7.

Table 7: Flow properties of Solid SEDDS.

Batch	Bulk density (gm/ml)	Tapped density (gm/ml)	Hausner's ratio	Carr's index	Angle of repose
LP1	0.77	0.75	1.1	9.09	27.75
LP2	0.81	0.76	1.06	15.71	29.05
LP3	0.76	0.65	1.26	7.89	27.14
LP4	0.81	0.70	1.09	13.58	26.56

Effect of dilution on solid SEDDS

The formulation LP4 has found to have "Good" dilution than that of other formulations.

In-vitro dissolution test

In-vitro drug released was performed between marketed formulation and optimized batches. The in-vitro dissolution of prepared solid SEDDS was compared with marketed formulation (LOSAR®). From the result it was observed that LP4 shows more release compared to the marketed formulation these findings conclude an enhancement of permeability and, as a result the improved bioavailability of Losartan potassium.

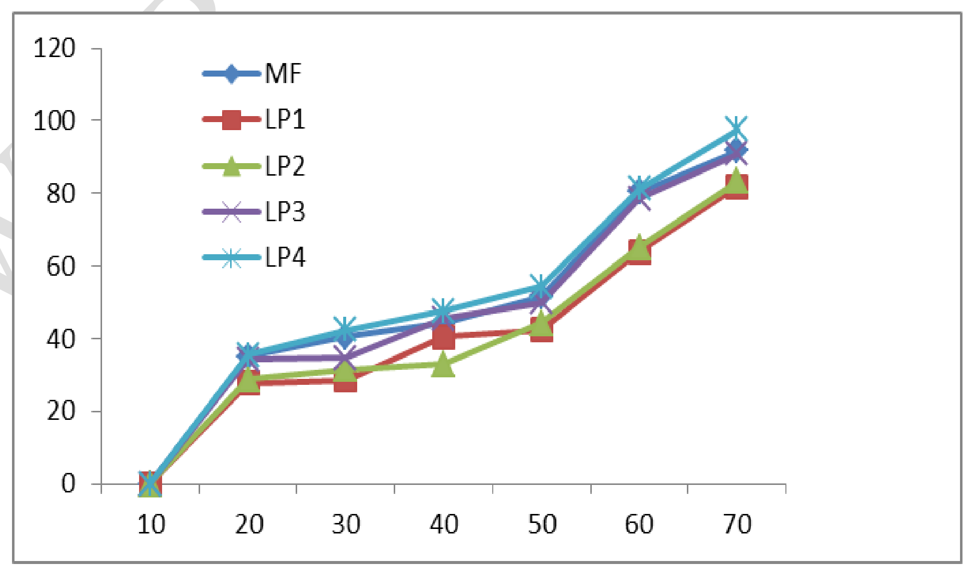

Fig. 11: In-vitro drug release.

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Drug excipient interaction by ATR-FTIR

From the observation of all FTIR spectra, it was evident that all important peak of losartan potassium and the excipients used were located in the solid SEDDS. Hence it could be concluded that there was not any chemical interaction between the drug and excipients.

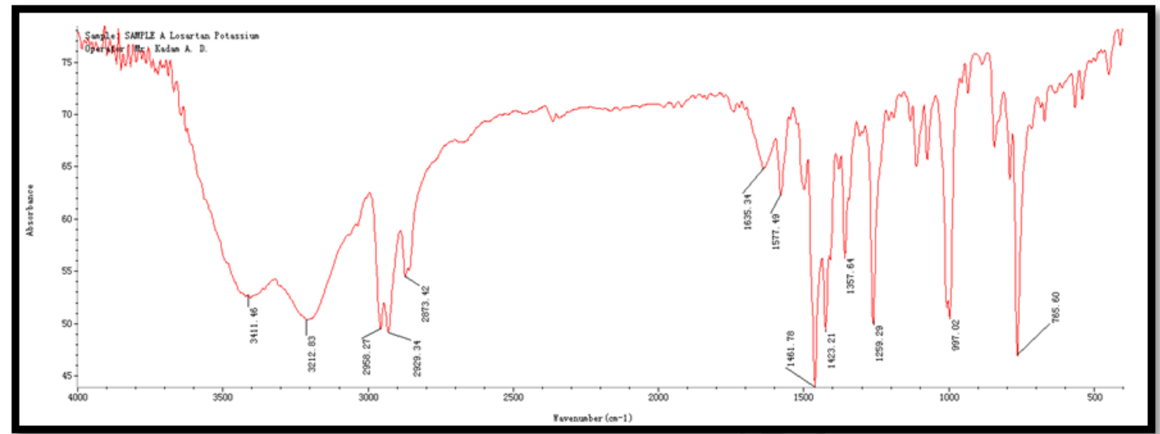

Fig. 12: Infrared spectrum of Losartan potassium.

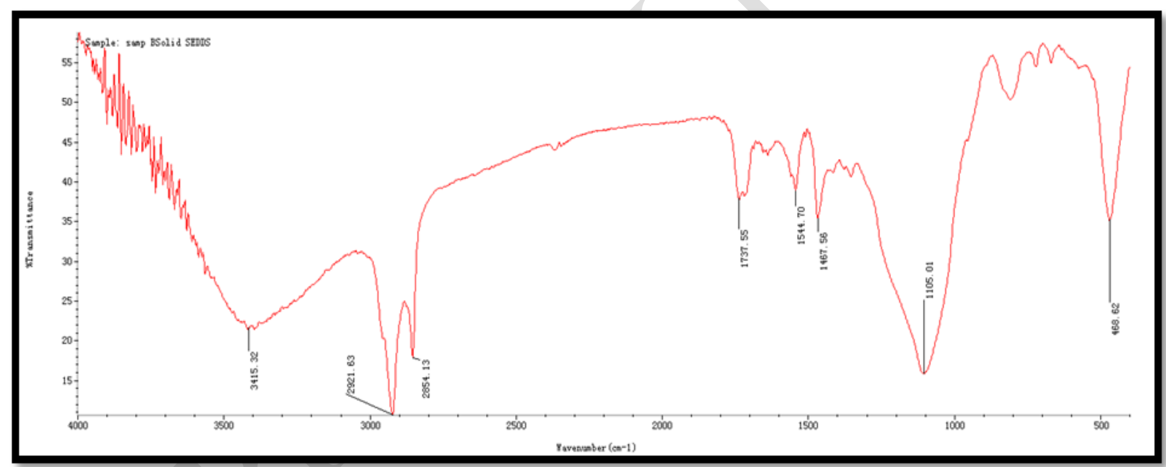

Fig 13: Infrared spectrum of solid self emulsified drug delivery system.

CONCLUSION

Liquid SEDDS of losartan potassium with oleic acid as oil phase, SDC-PC as surfactant and PEG400 as co-surfactant was successfully developed. Based on thermodynamic stability study, self emulsification time, % transmittance, zeta potential, particle size study LP4 formulation was selected. Based on above studies it was concluded that SDC-PC can be used as surfactant in SEDDS which shows good results. So LP4 formulation further converted to solid SEDDS using Aerosil 200 as a carrier and evaluated for the flow properties, effect of dilution, in-vitro drug release and FTIR studies. % drug release of solid SEDDS was almost similar to marketed formulation LOSAR®.

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ACKNOWLEDGMENT

I would like to convey my sincere thanks to department of pharmaceutical science, YSPM's YTC, DBATU, for excellent laboratory facilities necessary for carrying out this work. I would like to thank Nikhil chemicals, satara and Dolphine pharmacy Pvt. Ltd. Mumbai. for providing samples as a vehicles. Losartan potassium was obtained from YARROW CHEM PRODUCTS, Mumbai was duly acknowledged.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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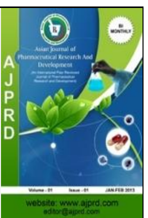

Research Article

Evaluation of Antiepileptic Activity of Ficusracemosa in Chemicals Induced Epilepsy in Mice

Prajakta J. Chavan *, Atish B. Velhal.Vithal J Chawre, Vivek K. Redasani

Department of Pharmacology ,YSPM's Yashoda Technical Campus Wadhe ,Satara , India.

A B S T R A C T

Objective: To Evaluvate of Antiepileptic Acivity of Ficus Racemosa Extract Against Chemicals Induced epilepsy in mice, ficusracemosa is also used as antihyperglycemic, antiinflammatery , hepatoprotective action. **Method :** Anticonvulsant activity of three distinct dose levels of ethanolic extract of Ficus racemosa (100, 200, and 400 mg/kg) was tested in Swiss albino mice in seizures induced by Pentylenetetrazol (PTZ). Statistical analysis was carried out by one-way analysis of variance followed by Dunnett's test. **Results**: The presence of , flavonoids, were detected in the bark of Ficus racemosa. The extract dose-dependent effect in the delay of the onset of seizures and reduction in the duration of seizure. **Conclusion :** The ethanolic extract of Ficus racemosa exhibited significant and dose-dependent antiepileptic activity, which may be due to the presence of antioxidant principles like flavanoidsa nd other phytoconstituent produce protective activity against PTZ.

Keywords: Pentelynetetrazole, Anticonvulsant activity, diazepam, *Ficus racemosa* bark.

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***Address for Correspondence:**

Prajakta J. Chavan, Department of Pharmacology, YSPM's Yashoda Technical Campus Wadhe ,Satara , India.

INTRODUCTION:

Epilepsy is a chronic disorder of central nervous system, Epilepsy are disorders characterized by paroxysmal, abnormal, excessive or synchronous neuronal activity in the brain with 5-10% of the population. There are many antiepileptic agents available for treatment but associated with side-effects such as depression, ischemia, impaired cognition and motor disability. This made man to search for alternative medicine from natural source.

Medicinal plants used for the therapy of epilepsy in traditional medicine have been shown to anticonvulsant activities. which can be much cheaper and less time-consuming. Several useful medicines derived from plants have been discovered from scientific investigation of traditional claim.

Ficus racemosa is also known as F. Glomerata. Ficus racemosa has various synonyms like Udumbara (U gular etc. It is used in treatment of burning sensation and obesity, anti-ulcer antipyretic antidiabetic diuretic, like astringent, and useful in vaginaldisorder Epilepsy is the term used for a group of disorders characterized by recurrent spontaneous seizures and involves hyperexcitable neurons. It is assumed that there is an imbalance between inhibitory GABA-mediated and excitatory glutamate-mediated neurotransmission. It is commonly associated with the brain.

The seizure activity during epilepsy decreases the antioxidant defense mechanism in the brain and increases the amount of free radicals, which further induces the oxidative stress. Free radicals (FR) can be defined as molecules or molecular fragments that contain one or more unpaired electrons. These free radicals were involved in causation of lipid peroxidation, brain edema and epilepsy.

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Material and Methods:

Drug andchemicals

The standard drugs of Diazepam were obtain form (Ranbaxy), Pentylenetetrazole obtained from (OZONE® INTERNATIONAL (INDIA) , All other chemicals used were of analytical grade.

Plant collection:

The bark of ficusracemosa plant were collected and shade dried and made in coarse powder

After collection Ficus racemosa bark were cleaned, washed to remove any dirt, dust and foreign particles. Botanical identity of plant specimen was authenticated by Dr. S. A. Mohite, Head, Department of Botany, Lal Bahadur Shastri College, Satara (MS), India. A voucher specimen of the bark has been deposited in the department for future reference .The bark were coarsely powdered and further utilized for preparation of ethanol extract.

Plant Extraction:

The ethanol extraction of bark of Ficus racemosa was carried out by soxhlet apparatus. The bark were crushed and ground to powder and placed into extractor. The ethanol was poured on powder with three cycles. After that extraction process was started and continued till appearance of solvent in syphon tube turns brown to clear. Then brown colored solvent mixture from round bottom flask was collected and evaporated with the help of rotary evaporator to get a solid residue. The residue was placed in a vacuum desiccator and was further used for the experiments.

Pharmacological Investigation:

Experimental Animals: Adult Swiss albino mice (25-30 g) were used for this study. The animals were housed at 24°C ± 2°C and relative humidity 55 ± 5 with 12:12 h light and dark cycle. They were provided food and water *ad libitum*. The experimental protocol was approved by the Institutional Animals Ethics Committee of Yashoda College of Pharmacy, Satara , Maharashtra.

Acute toxicity study:

The acute oral toxicity was performed as per the Organization for economic co- operation and development (OECD) guideline 423.[14]Acute toxicity study was performed in Swiss albino mice. The animals were grouped with three numbers in each were administered orally with the ethonolic extract of Ficus racemosa was given to animals with starting dose 300mg/kg in 0.1% CMC for first According to observations of first group, study was carried out further on next group with dose 2000 mg/kg. From obtained results it was clear that no death as well as no toxicological signs in animals so, for confirmation of safety of extract study was repeated with dose 2000mg/kg on third group. After administration of extract, animals were observed carefully for first 30 min and periodically for 24 h with special attention during first four hours. Animals were further observed daily for subsequent 14 days. Effects such as changes in skin fur, eyes and mucous membranes were observed daily. Animals were further observed for salivation, diarrhea, tremors, lethargy, convulsions, sleep, and coma. The parameters like body weight, food, and water intake were checked periodically every two days[20].

Evaluation of antiepileptic activity:

PTZ-induced convulsions in mice

Swiss mice of either sex were randomly divided in to Five different groups of six mice each. Group I received the vehicle, Group II received the standard drug, Diazepam at the dose of 5 mg/kg, i.p. Group III, IV and V received EEFR at the doses of 100, 200 and 400 mg/kg, p.o. respectively. Group I mice were administered with PTZ (80mg/kg, i.p.) 1 h after vehicle. Group

II mice received PTZ 30 min after Diazepam (5 mg/kg, i.p.). Group III,IV and V mice received different doses of plant extracts, p.o. 1 h before PTZ. Onset time as well as duration of convulsions were recorded [12].

Statistical analysis

The data were analyzed using one-way analysis of variance, followed by Dunnett's test. $P < 0.05$ was considered as sta1tistically significant. The data are expressed as mean ± standard deviation.

RESULT:

Preliminary phytochemicalinvestigation

Table 1.Shows the findings of qualitative analysis of Preliminary phytochemical screening of Ethanolic extract of ficusracemosa bark indicated the presence of steroids, triterpenoids, polyphenolics, coumarins, flavonoids and tannins, while alkaloids and saponins were absent.

Table 1. Qualitative analysis of the phytochemicals inextracts of *Ficus racemose bark*

Phytoconstituent	EEFR
Alkaloid	-
Carbohydrate	+
Protein	–
Steroid	+
Flavonoid	+
Tannin	–
Saponin	–
Lipid	+

+ indicating Positive , - indicate negative and EEFR indicate Ethanolic Extract of ficus Racemosa

PHARMACOLOGICAL INVESTIGATIONS

Acute toxicitystudy

The acute toxicity study began with a 300mg/kg starting dose. During a 14-day observation period, oral administration of a 300 mg/kg dosage of ethanol extract of *ficus racemosa bark* caused no significant toxicity.

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From above results it is clear that given dose was safe and hence further study was performed by administering 2000mg/kg dose of extract to next group of animals. There were no indicators of toxicity and mortality [Table 2.], as well as the animals' morphological characteristics and general appearance did not change. There was no salivation, diarrhoea, tremors, convulsions, lethargy or unusual behavior observed during study in treatment group. For further confirmation of results effect was checked by giving same dose (2000mg/kg) to another group of three animals and results parameters were normal. The oral LD50could be over 2000mg/kg body weight. As a result, greater dose testing of the extracts may not be necessary, and the extracts were practically non-toxic.

Table 2: Effect of *Ficus racemosa bark* extract for sign of toxicity and mortality (n = 3).

Group	Treatment	Sign of toxicity (ST/NB)	Mortality (D/S)
Normal Control	Vehicle	0/3	0/3
Aqueous extract	2000 mg/kg	0/3	0/3
Alcoholic extract	2000 mg/kg	0/3	0/3

STs = Sign of toxicity, NB = Normal behaviour, D = Died, S = Survived.

Table 3: Effects of ficus racemose bark extract dose 2000mg/kg on morphological characteristics and general appearance in mice (n=3)

Sr. No.	Response	Before	After
1.	Alertness	Normal	Normal
2.	Touch response	Normal	Normal
3.	Torch response	Normal	Normal
4.	salivation,	Normal	Normal
5.	Diarrhoea	Absent	Absent
6.	Tremors	Absent	Absent
7.	Convulsions	Absent	Absent
8.	Lethargy	Absent	Absent
9.	Skin fur	Normal	Normal
10.	Pinna reflux	Normal	Normal
11.	Corneal reflux	Present	Present
12.	Pupils	Normal	Normal
13.	Lacrimation	Normal	Normal
14.	Gripping strength	Normal	Normal
15.	Urination	Normal	Normal
16.	Hyper activity	Absent	Absent

PTZ induced Epilepsy

The average time of onset, duration of convulsions and percentages of inhibition of convulsions were presented in table. 3 EEFR treated mice not only exhibited delay in the onset time of convulsions at the doses of 100, 200 and 400 mg/kg, p.o. but also showed reduced duration of convulsions when compared with the control group mice.

All the three doses of EEFR afforded significant protection in a dose-dependent manner against convulsions induced by PTZ ($P < 0.01$). Animals pretreated with EEFR at all the three doses exhibited significant antiepileptic activity and more percentage of inhibition of convulsions when compared with Diazepam treated animals.

Table 3: Anticonvulsant effect ethanolic extracts of Ficus racemosa on PTZ-induced convulsions in mice.

Experimental Group	Dose	Onset of convulsion(min)	Duration of convulsion(min)	Mortility	% Protection
Control	Vehicle	1.05.±0.12	.4.30 ± 0.20	6/6	0%
Standard (Diazepam)	5mg/kg	0.000.±0.00	0.00.±0.00**	0/6	100%
EEFR	100mg/kg	2.5.±0.17	2.9 ±0.40	4/6	33.33%
EEFR	200 mg /kg	3.1.±0.22	1.8± 0.03	3/6	50%
EEFR	400mg /kg	4.5.±0.25	1.2.± 0.004	2/6	66.66%

Value are mean ± SEM ;n= 6; ANOVA followed by Dunnets test. Where *p< 0.05 **p<0.01 and***P<0.001

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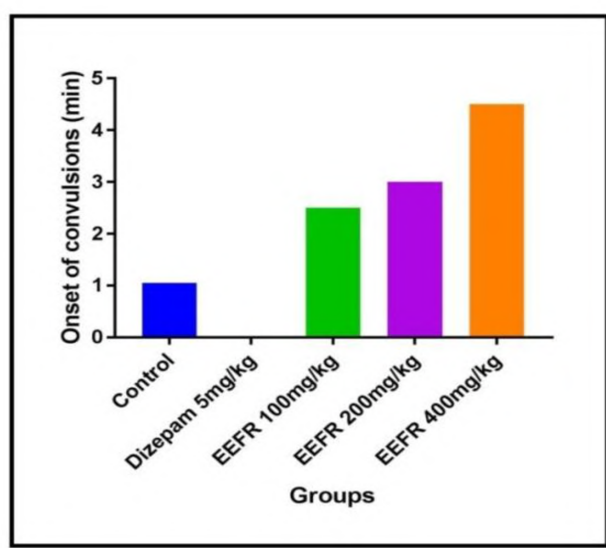
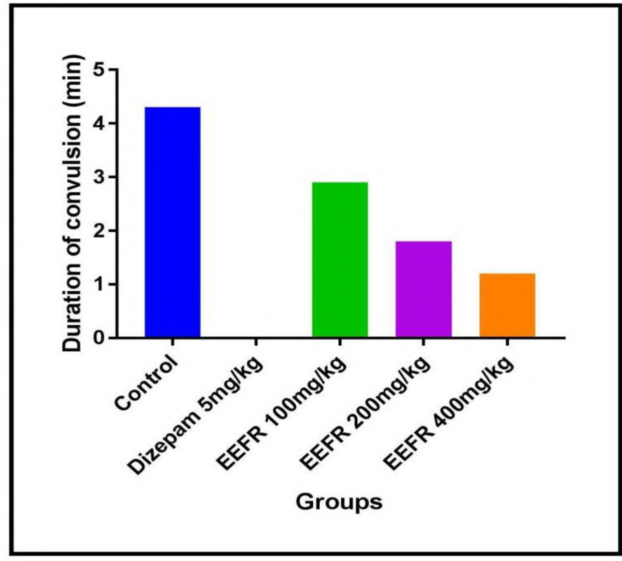


Graph 1.:Effect of EEFR on onset of convulsions in PTZ induced convulsions

Values represent mean ± SEM; n=6; Analysis was performed using one way ANOVA followed by Duneet multiple comparison test; p value less than 0.05 was considered as statistically significant. $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$; ##Data compared with control



Graph 2 : Effect of EEFR on Duration of convulsions in PTZ induced convulsions

Values represent mean ± SEM; n=6; Analysis was performed using one way ANOVA followed by Duneet multiple comparison test; p value less than 0.05 was considered as statistically significant. $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$; ##Data compared with control.

DISCUSSION:

Epilepsy is one of the chronic and most common neurological disorders. The basic and major mechanisms associated with epilepsy are increased synaptic connectivity of neurons (such as excitatory glutaminergic neurons), (weakening of potassium channels and/or inure persistent sodium channels, changes in voltage-gated ion channels), perturbance in synaptic receptors (suppressed GABAergic receptor altered nicotinic receptors), decrease in inhibitory neurotransmission (decreased GABA levels), enhanced excitatory neurotransmission (enhanced glutamate levels. [29]

In this study, anticonvulsant activity of bark extracts of Ficus racemosa PTZ-induced convulsions in Swiss albino mice.

PTZ is a potent GABA receptor antagonist, it is well known to decrease the GABA levels, and density of GABA A receptors in various parts of the brain, this leads to continuous stimulation of cortical neurons and results in convulsions similar to absence seizures is human Hence, if thought that the agents which enhance GABA levels, GABA-A receptor agonists (like diazepam), the agents behave like GARBA are thought to be useful in abolishing PTZ-induced convulsions [6].

The reports on chemical constituents of EEFR have shown the presence of antioxidant and chemopreventive principles namely, racemosic acid, bergenin, tannins, kaempferol, rutin, bergapten, psoralenes, coumarin and phenolic glycosides antioxidant used in Parkinson's ,epilepsy, Alzheimer [26][1] .

PTZ-induced convulsions in mice are a suitable model for petit mal epilepsy. PTZ is GABA antagonist. This assay has been used primarily to evaluate AED. Drugs which antagonize PTZ- induced seizures are generally useful in petit mal epilepsy. It has been indicated that PTZ- induced seizures can be prevented by drugs that reduce T-type Ca^{2+} currents, such as ethosuximide and also by drugs that enhance GABAA receptor-mediated inhibitory neurotransmission, such as benzodizepine .

It is also found that many flavonoids could act as benzodiazepine-like molecules in the central nervous system and modulate GABA-generated chloride currents in animal models of anxiety, sedation and convulsion [34][4].

The average time of onset, duration of convulsions and percentages of inhibition of convulsions were presented in EEFR treated mice not only exhibited delay in the onset time of convulsions at the doses of 100, 200 and 400 mg/kg, p.o. but also showed reduced duration of convulsions when compared with the control group mice. All the three doses of EEFR afforded significant protection in a dose-dependent manner against convulsions induced by PTZ ($P < 0.01$). Animals pretreated with EEFR at all the three doses exhibited significant antiepileptic activity and more percentage of inhibition of convulsions when compared with Diazepam treated animals. convulsion were induced the all animals by given PTZ 80 mg/kg i.p. 100% mortality was observed in control groups. Diazepam at the dose of 5 mg/kg p.o. significantly delayed onset of convulsion and decreased duration of convulsion and also delayed onset of convulsions and decreased duration of convulsions and also prototected 100% mortality rate.

CONCLUSION:

Based on the above investigations, it may be concluded that the ethanolic extract of bark of ficusracemos*a* exhibited significant antiepileptic activity. The presence of flavanoids may partially contribute the significant

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activity of EEFR by enhanced GABAergic neurotransmission which responsible for the antiepileptic effect.

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RESEARCH ARTICLE

ROLE OF FUNCTIONALIZED GUAR GUM IN SOLID DISPERSION OF NON-STEOIDAL ANTI-INFlAMMATORY DRUG

S. P Alane, A.B. Velhal and Dr. V.K Redasani
Department of Pharmaceutics, YSPM's Yashoda Technical Campus, Satara, Maharashtra State, India.

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Abstract

The current investigation was developed to study the role of functionalized guar gum as carrier in solid dispersion of iboprofen. The solid dispersion technique using aminated guar gum would be an effective approach for increasing the solubility and increasing dissolution behaviour of ill fathomable medicament than the native guar gum. The results of FTIR and DSC studies confirmed that there is no chemical interaction or no incompatability between the drug and excipients. The invitro dissolution study was performed for the the prepared formulations. Based on the results SD3 was shown highest drug release 99.41% within 24hrs. Stabiliy study was conducted as per ICH guidelines and the falloutsrevealed that there is no physical or chemical change.it may be concluded that solubility of ibuprofen can br improved by using functionalized guar gum in the solid dispersion, which provides a wide scope for the therapeutic efficiency.

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Introduction:-

SolidDispersion: The term strong scattering alludes to a gathering of strong items comprising of somewhere around two parts, by and large a hydrophilic grid and a hydrophobic medication. The lattice can be either glasslike or nebulous. The medication can be scattered microscopically, in nebulous particlesorincrystallineparticles.[1]

Oral availability of medication relies upon its solvency or potentially disintegration rate, in this way serious issues related with these medications was its very dissolvability in natural liquids, which results into helpless bioavailability after oral organization. Numerous techniques are accessible to further develop disintegration rate, dissolvability attributes, including salt arrangement, micronization and expansion of dissolvable or surface dynamic specialists. The term strong scattering alludes to a gathering of strong items comprising of somewhere around two parts, by and large a hydrophilic network and a hydrophobic medication. The lattice can be either glasslike or nebulous. The medication can be scattered microscopically, in formless particlesorincrystallineparticles.2Strong scattering is one othese strategies, which was most broadly and effectively applied to work on the solvency, disintegration rates and thus the bioavailability of inadequately solvent medications. The idea of strong scatterings (SDS) was presented in 1961 by Sekiguchi and Obi, in which the medication is scattered in inactive water-dissolvable transporter at strong state. Several water soluble carriers such ashydroxyl propyl methyl cellulose, ethyl cellulose, beta cyclodextrin, urea, lactose,citric acid, poly vinyl pyrrolidone(PVP) and poly ethylene glycols such as carriersforSolid dispersion

Corresponding Author:- Sarika Alane
Address:- Department of Pharmaceutics, YSPM's Yashoda Technical Campus, Satara, Maharashtra State, India.

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Yashoda Technical Campus
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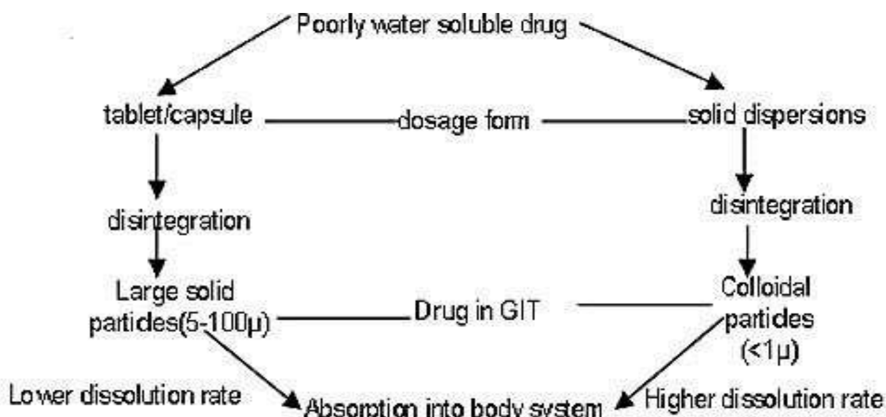

Figure 1:- Schematic representation of the bioavailability improvement of ineffectively water solubledrugbysolid dispersion technique.[4]

Naturalcarrier

More hydrophilic nature of carriers enhances the faster release of drugsfrom solid dispersion. A poor water soluble or insoluble carriers may leads toslowerreleaseof drug

Guar gum is a galactomannan, obtained from plant Cyamopsis tetragonolobus. Powder is whitish and yellowish consisting of slight odor. Guar gum is mainly consisting of thehigh molecular weight polysaccharides composed of galactomannans which are consisting of a linear chain of (1→4)-linked β-D-mannopyranosyl units with (1→6)-linked α-D-galactopyranosyl residues as side chains. The mannose: galactose proportion is roughly 2:1. The atomic weight territory is 50,000-8,000,000.

Amination of Natural gums

Recently,chemical modification or derivatization of natural polysaccharides hasbeen reportedto improve the functional propertiesof native gums.Reports in the literature suggestthatthe derivatives of polysaccharides (amine, thiol, carboxymethyl) can be employed tomanipulateswelling, bioadhesion and drugrelease A couple of instances of polysaccharide subsidiaries previously revealed in writing incorporate N-(2-hydroxy) propyl-3-trimethyl ammonium chitosan, glycol chitosan, n-succinyl chitosan, thiolated hydroxyl ethyl cellulose carboxymethyl tama-rindkernelpowder,aminated tamarindkernel polysaccharide, thiolatedchitosan.

Materials and Methods:-

Chemicals Used

Ibuprofen, Guar gum, Ethylene diamine, Sodium Bromide, Ethyl alcohol procured from Loba Chem. Mumbai, India.

Instruments Used

AnalyticalBalance (Shimadzu, Japan.), FTIRSpectrophotometer (JascoFT-IR8201PC), DissolutionApparatus (Electro labTDT-08L), DifferentialScanningCalorimetry (PerkinElmer,Pyris6DSC,Germany), UVSpectrophotometer (U.V 1700 Shimadzu, Japan.) and Hotairoven (Thermolab)

Preformulation Studies

Construction ofStandard GraphofIbuprofeninpH7.4Phosphatebufferbyusing theUVmethod

100mg of ibuprofen was weighed and transfer into a 100ml of volumetricflask and was dissolved in phosphate buffer of pH7.4 and make up to 100ml.Thiswas the standard stock solution containing 1mg/ml of ibuprofen.From this stock arrangement, 10ml was taken and made up to 100ml with phosphate cushion pH 7.4.This was the second standard stock solution (100µg/ml).From this solutiondilutionsof10µg/ml,20µg/ml,30µg/ml,40µg/ml,50µg/mlweremadeandabsorbance wasmeasured at 222nm.

Synthesis of Functionalized Guar gum

AminationofGuargum

In 3000ml water add 60gm of Natural Guargum. To this solution add aminating agent ethylene diamine (25ml) with continuous stirring at constant temperature (20-60^{0}C) for 6 hr. then slowly add reducing agent Sodium Bromide ($NaBH_4$) for 2hrs until formation of thick gel. Wash this gel several times with ethyl alcohol and collect the precipitate of aminated derivative of Guar gum.

PreFormulationStudies

Appearance

Colour and physical state of the drug is done by Visual examination.

Melting point

Melting point of the Ibuprofen was resolute by capillary method in triplicate.

Solubility determination study

The dissolvability of Ibuprofen was dictated by the harmony dissolvability technique in which a soaked arrangement of the material was gotten by blending an overabundance of medication in a steady amount of dissolvable until immersion or balance was accomplished in a vortex blender. Then, at that point it was separated through Whatman channel paper (no.1) and focus was investigated by UV spectrophotometer at 222 nm. The dissolvability of not really set in stone in refined water and pH across the gastrointestinal plot, for example in pH 1.2, 6.8, and 7.4.

DifferentialScanningCalorimetry(DSC)

Thedifferentialscanningcalorimetry(DSC)ofpuredrugs,soliddispersion,andthephysicalmixtureoftheremedy wasperformedusingDSCinstrument (Perkin Elmer Pyris 6 DSC, Germany), for the measurement of heatloss or gain resulting from physical or compound changes inside the example as a component of temperature. Around 6-7 mg of the example was made an appearance aluminum DSC container and hermetically sealed with aluminium lids. An underlying incline was utilized to hop the temperature to 30ºC and afterward a steady warming pace of 10ºC/min was utilized something like 400ºC under nitrogen temperature.

FourierTransformInfrared(FTIR)studies

The similarity of medications and excipients utilized under trial condition were contemplated. The examining range was 400 to 4000 cm-1 and the goal was 1cm-1. This unearthly examination was utilized to check the similarity of medications with the excipients utilized and put away.

Powder X-ray diffraction

Powder X-beam diffraction studies were performed to check for any crystallinity in the definition after it was made and after the strength studies were performed. Staying away from recrystallization of the medication in the definition was one of the objectives of the current investigation. Skillet logical X-Pert Pro V1.6 with X Pert Data Collector V2.1 programming was utilized furnished with a CuKa2 anode cylinder and diffractometer of span 240 mm. The X-beam powder diffraction check was performed utilizing a BB004 level stage. The powdered example was put in an aluminum test holder that had a 2.5 cm square with a profundity of 0.5 mm. The information were gathered by filtering the example at 45 kV and 40 mA. Tests were filtered from 5 to 50°C 2θ at a stage size of 0.0170 and output pace of 1.0°C/min.

FormulationDevelopment

PreparationSoliddispersionofIbuprofen

Preparation of solid dispersions of Ibuprofen is to improve the solubility ofIbuprofen and dissolution rate. Solid dispersion of Ibuprofen was prepared byhot melt method. The drug and carrier were mixed in 1:1, 1:2 and 1:3ratios in ethanol. Solventwas removedby evaporation under reduced pressureThe mass was crushed and gone through strainer no 60

PreparationofphysicalmixturecontainingIbuprofen

Thecorporeal assortments of Ibuprofen-guar gum and Ibuprofen Aminated guar gum in the identicalheft ratio (1:3) were primed by scrupulously mixingthe appropriate amount of two components for 10 min in a mortar. The concoctionsweresievedthrougha60meshscreenandstoredinadesiccatorforfurtherevaluation.Solid dispersions were prepared by hot melt method. Correspondingphysical mixtures were heated in an oil bath at 175^{0}Cuntil they melted.

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Solidification was reached by cooling to room temperature under ambient conditions. Afterwards, the mixture was pulverised, sieved, and thefraction≤160μm wasselected.The methodofpreparationandcompositionwere givenTable1.

Table 1:- Composition of various batches of Physical mixturesandIbuprofen SolidDispersion.

BatchCode	Composition	Ratio
S.D1	Ibuprofen:Aminated guar gum	1:1
S.D2	Ibuprofen:Aminated guar gum	1:2
S.D3	Ibuprofen:Aminated guar gum	1:3
S.D4	Ibuprofen:guar gum	1:1
S.D5	Ibuprofen:guar gum	1:2
S.D6	Ibuprofen:guar gum	1:3

EvaluationofIbuprofensoliddispersionsandcorporeal mixtures
Evaluationstudieswerecarriedoutbyestimatingdrug contentand*invitro*dissolutionstudies.

Determination of solubility of various solid dispersions
Ibuprofen laden strong scatterings, actual combinations, and unadulterated Ibuprofen identical to 30 mg were gauged and moved to four cups containing 50 mL of refined water, pH 1.2 acetic acid derivation cushion, phosphate support pH 6.8, and phosphate cradle pH 7.4. The example was fomented at 80 rpm in a thermostated shaking water shower at 37±0.5°C for 8 h. The supernatant arrangement was then sifted through Whatman channel paper. The filtrate was weakened and the absorbance was estimated utilizing an UV-Vis spectrophotometer.

Drugcontent
The drug content of each solid dispersion physical mixture weredetermined by UV-spectrophotometry. Accurately weighed quantity of samplesfrom all batches equivalent to 100 mg of Ibuprofen was transferred to a 100mlvolumetric flask containing 100ml of phosphate buffer pH 7.4 and the absorbancewasmeasured at 222nm.

Invitrodissolutionstudies
Thepreparedsoliddispersionswereaccuratelyweightequivalentto100mgofthedrug.Thesesoliddispersionsarefilledinemptycapsulesand analysed for drug release in 900ml of phosphate buffer pH (7.4) as dissolutionmedium at 37± 0.5ºC and 50rpm. 5ml of the sample solution was taken from thedissolutionapparatusandthesamevolumereplacedwithfreshdissolutionmediumatpredeterminedtimeintervalsfor5min.Theabsorbanceofthesesolutionswasmeasuredat 222nmusingUV-Visiblespectrophotometer.

Stabilitystudies
Stability study was carried out to observe the effect of temperature andrelative humidity on selected formulation (SD3), by keeping at 40±2ºC, in air tighthighdensitypolyethylenebottlesforsixmonths,atRH75±5%.Physicalevaluationwascarried out ineach month.[9]

Table2:- ICHguidelinesforStabilitystudy.

Study	Storagecondition	Timeperiod
Longterm	25ºC±2ºC/60%RH±5%RH	12month
Intermediate	30ºC±2ºC/65%RH±5%RH	6 month
Accelerated	40ºC±2ºC/75%RH±5%RH	month 6

Results and Discussion:-

PreparationofCalibrationcurveforIbuprofen

Table 3:- CalibrationcurveofIbuprofen.

Concentration(μg/ml)	Absorbance(222nm)
0	0
10	0.151
20	0.351
30	0.521
40	0.713
50	0.912

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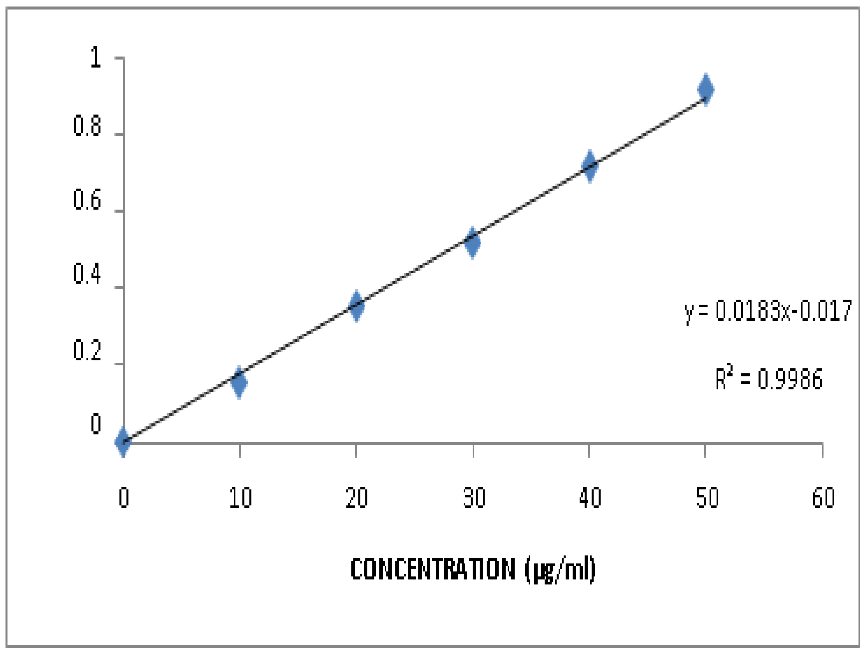

Figure 2:- Calibrationcurveofibuprofen.

The calibration curves were linear and obeyed Beer-Lambert's law in theconcentration range 10-50µg/ml. The correlation coefficient values were 0.9986indicatingexcellent linearityof thedata.

Appearance

Ibuprofenappeared as crystalline solid.

Melting point

Melting point of drug was determined by capillary method. The result is found to be 75-77^{0}C.

Saturation solubility

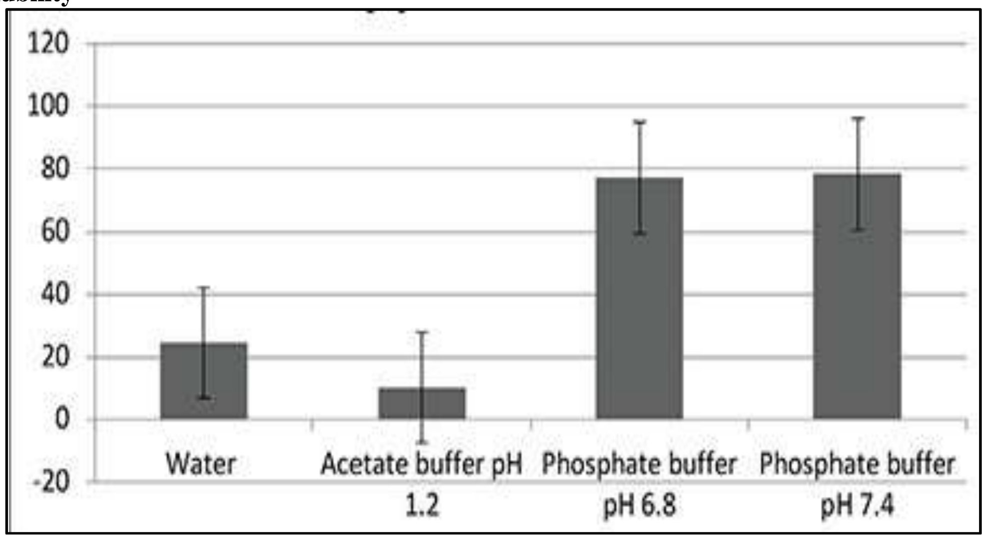

Figure 3:- Graphical representation of Solubility data of Ibuprofen in different solvents.

The solubility data of Ibuprofen in distilled water, Acetate buffer pH 1.2, phosphate buffer pH 6.8, and phosphate buffer pH 7.4 at 25°C are given in Table 11. The comparison of Ibuprofen in different solvents is presented graphically in Figure 15. From the Results we can conclude that the drug is poorly soluble in nature. So is suitable for the formulation of Solid disperssion toenhance itssolubility.

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CompatibilityStudies
DifferentialScanningCalorimetry(DSC)

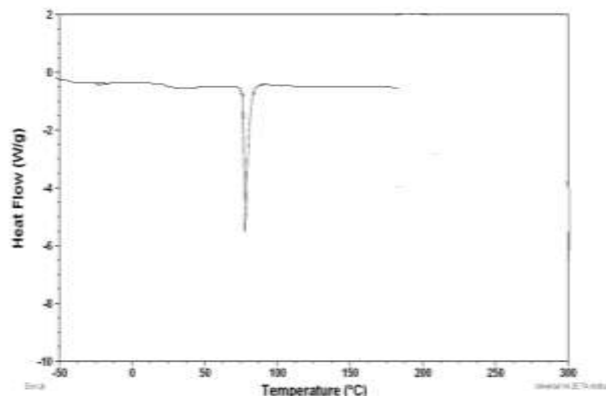

Figure4:-DifferentialScanningCalorimetryofIbuprofen

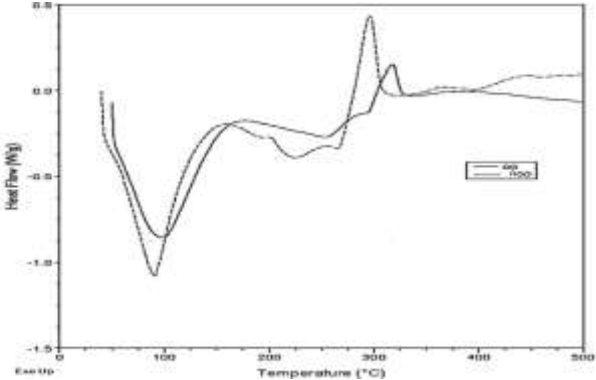

Figure5:-DifferentialScanningCalorimetryofGuar gum(GG) and Aminated guar gum(AGG).

The DSC thermogram of pure Ibuprofen showed a sharp endothermic peakat 100.00°C which corresponds to its melting point.Observation revealed that the drug is pure without any impurities.SC thermogram shows the thermal behavior of native and aminated guar gum. For native guar gum, endothermic peaks were detected at 253 and 296 ◦C, and exothermic peak was detected at 317 ◦C. Aminated guar gum showed endothermic peaks at 223 and 274 ◦C, and exothermic peak at 295 ◦C. All endothermic and exothermic peaks for both guar gum samples are shown in the above figure.

Fourier TransformInfraredStudies
The FTIR Spectra of Ibuprofen in pure form and theirphysical mixturewas observed, the result showed that there is no interaction between drug andpolymers. From the FTIR spectral Figures 6 to 10 Interpretations the followingresult was obtained. The FTIR of Ibuprofen and combinations of polymers showsintense bandin thetable7 to 10asfollows.

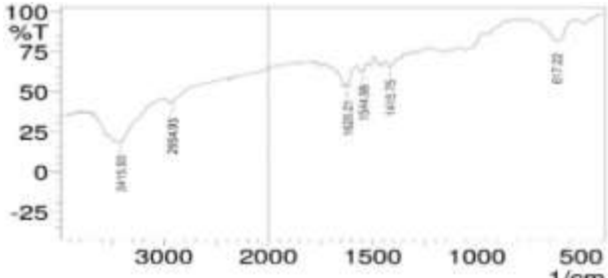

Figure 6:- FTIR Spectrum of Ibuprofen.

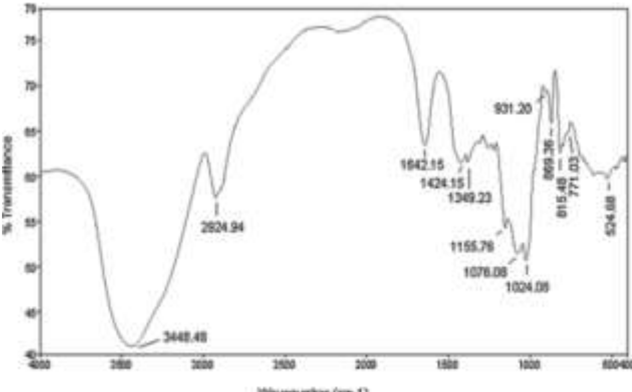

Figure7:- FTIRSpectrumofGuar Gum.

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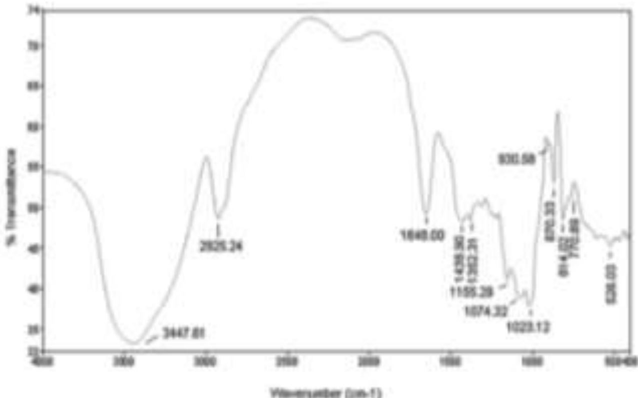

Figure 8:-FTIRSpectrumofIbuprofen+Guar gum.

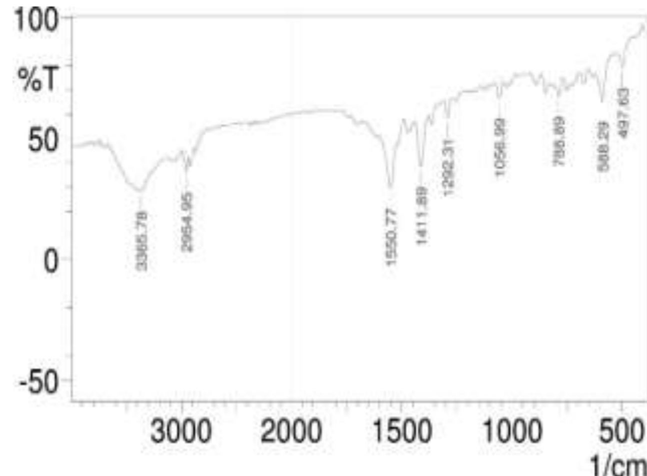

Figure 9:- FTIR Spectrum of Ibuprofen+ aminated Guar gum.

Powder X-ray diffraction (XRD)

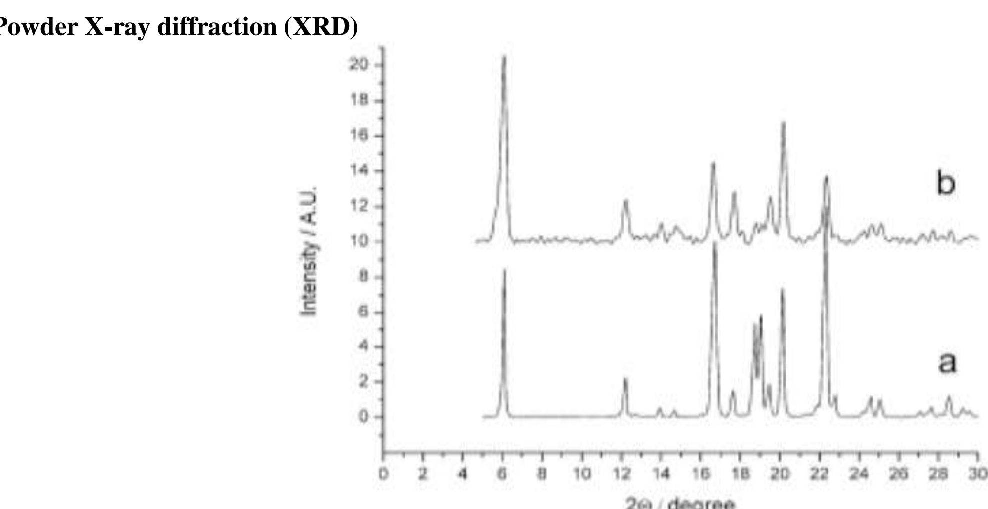

Figure 10:- XRD Graph Of a) Pure Ibuprofen and b) Ibuprofen in Optimized Formulation SD3.

The powder X-ray diffraction patterns of solid disperssions and original powder are compared in Figure 9. Both XRDpatterns from Solid disperssion and original substance correspond to racemic ibuprofen,that is, the Solid disperssion structure a similar precious stone stage as the first substance. The little distinction in the pinnacle

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relative powers between thecurves a and b in the range of 17 < 2Ɵ < 21 is probably related to the difference in distribution of crystallographic orientations in the microsized original powder and Solid disperssion.

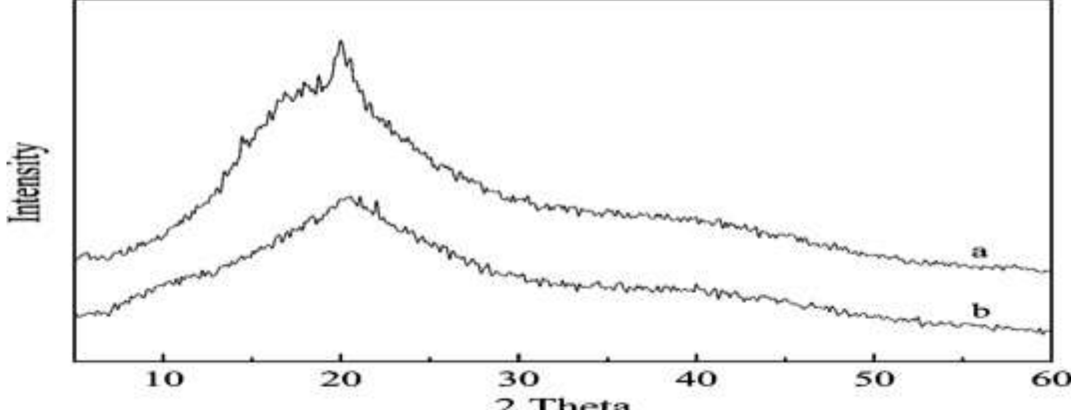

Figure 11:-XRD pattern of GG (a) and a representative AGG

The wide point X-beam diffractogram of local guar gum and an agent Aminated guar gum is introduced in Fig. 10. From Fig.10a, clearly local guar gum displays a tiny crystallinity. Comparable appearance has been accounted for local guar gum in the writing (Pal, Mal, and Singh, 2007). After Amination, an articulated decrease in crystallinity is noticed (Fig. 10b). This misfortune in crystallinity could be credited with the impact of the substitution of the Amine bunches by the Amination cycle. Amine bonds keep up with the dependability of guar gum gem, when they are broken, it could prompt diminishing the crystallinity.The drug content of each solid dispersion batch and physical mixture weredetermined byUV-spectrophotometrymeasured at 222nm.

Table 4:- Drug content of evaluation of solid dispersion containing Drug andCarriersfor various formulations.

Batchcode	%Drugcontent±S.D
S.D1	90±0.18
S.D2	95±0.78
S.D3	99±0.83
S.D4	91±0.74
S.D5	92±0.84
S.D6	93±0.74

Solubility Studies of Solid Disperssions of Ibuprofen

The solubility data of the physical mixtures containing Ibuprofen and guar gum, and Aminated Guar gum shown in Table 5. The solubility profile of the physical mixtures of Ibuprofen is shown infigure 12.

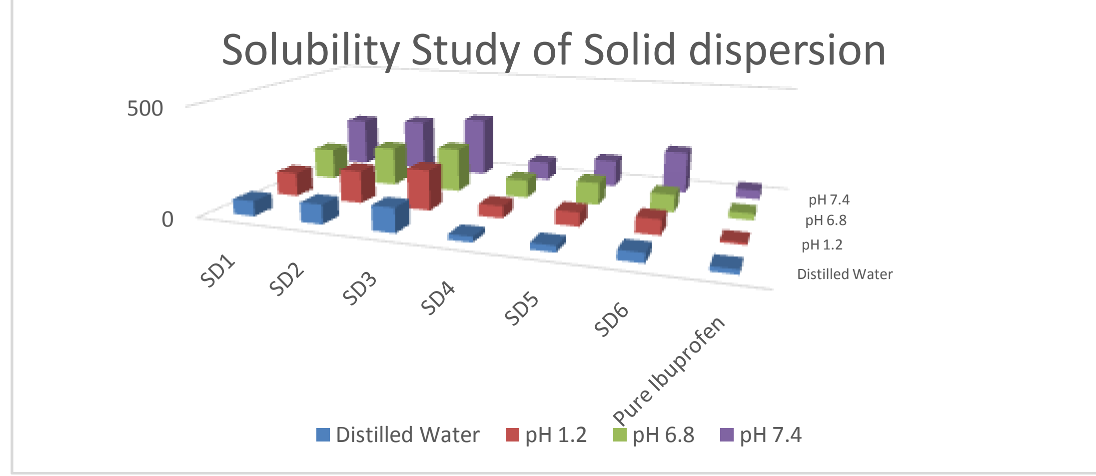

Figure 12 :-Solubility Studies of Solid Disperssions of Ibuprofen.

Table 5:- Solubility Studies of Solid Disperssions of Ibuprofen.

a) **Formulation Code**	b) **Distilled Water**	c) **pH 1.2**	d) **pH 6.8**	e) **pH 7.4**
f) SD1	g) 70±0.44	h) 110±0.60	i) 148±0.28	j) 234±0.20
k) SD2	l) 90±0.24	m) 150±0.60	n) 182±0.12	o) 250±0.92
p) SD3	q) 112±0.62	r) 186±0.36	s) 206±0.55	t) 284 ±0.86
u) SD4	v) 25±0.66	w) 56±0.97	x) 84±0.83	y) 88±0.83
z) SD5	aa)28±0.66	bb)64±0.24	cc) 104±0.28	dd)125±0.46
ee) SD6	ff) 42±0.61	gg)70±0.56	hh)82±0.38	ii) 196±0.84
jj) Pure Ibuprofen	kk) 20±0.49	ll) 10±0.86	mm)30±0.62	nn)42±0.24

As related to pure drug and, the solid dispersions prepared by Hot Melting Method showed higher solubility in Solid dispersions with Aminated Guar gum than the Solid Dispersions with Native Guar gum (Figure 12). The current examination proposed that this may be conceivable because of the planning of strong scatterings utilizing fluctuating convergences of normal transporters, which framed an eutectic combination and expanded the wettability of Ibuprofen and thus its solvency.

InvitrodrugreleasestudyofsoliddispersioncontainingDrugandcarriersfor variousformulations

Table 6:- Invitrodrugreleasestudy of solid dispersionontainingDrugandcarriersforvariousformulations.

Time(min)	**CumulativePercentageofDrugReleased±std dev.**						
	S.D1	**S.D2**	**S.D3**	**S.D 4**	**S.D 5**	**S.D6**	PI(Pure Ibuprofen)
0	0	0	0	0	0	0	0
5	10±0.11	13±0.15	12±0.13	10±0.16	10±0.11	12±0.12	0.005±0.32
10	12±0.12	22±0.37	20±0.32	23±0.22	20±0.54	24±0.45	1.009±0.03
15	16±0.24	30±0.22	23±0.63	31±0.34	28±0.22	29±0.27	3.024±0.21
20	20±0.14	40±0.68	30±0.24	52±0.18	35±0.33	32±0.36	4.043±0.052
25	27±0.23	52±0.35	36±0.16	70±0.16	46±0.56	38±0.24	7.19±0.35
30	33±0.88	60±0.54	48±0.34	74±0.14	50±0.38	46±0.28	10.361±0.42
35	38±0.54	67±0.18	52±0.25	79±0.18	63±0.13	56±0.44	11.54±0.07
40	42±0.82	70±0.17	67±0.23	84±0.20	68±0.26	64±0.83	11.892±0.09
45	50±0.24	72±0.19	78±0.26	88±0.23	74±0.16	73±0.56	13.892±0.12
50	63±0.56	80±0.17	80±0.48	90±0.18	84±0.22	86±0.38	15.892±0.26
55	72±0.58	86±0.19	92±0.16	93±0.27	89±0.43	90±0.22	18.892±0.32
60	80±0.88	90±0.38	98±0.32	96±0.30	93±0.26	95±0.32	20.892±0.71

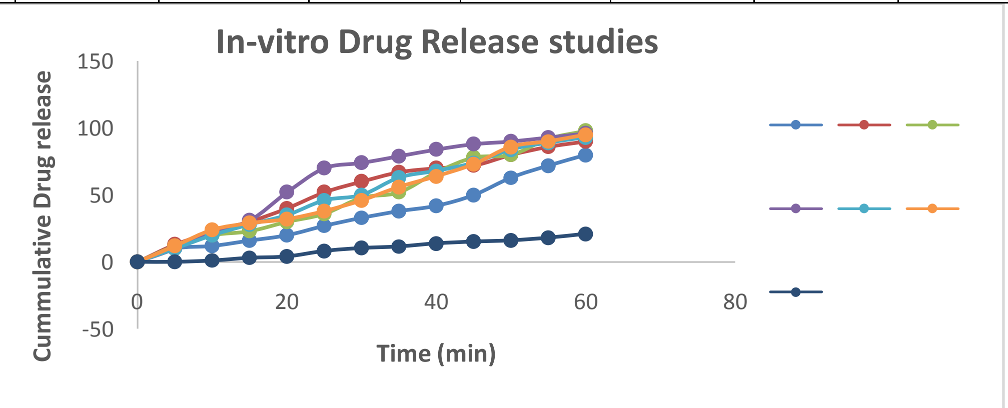

Figure 13:-In vitro drug release study of solid dispersion containing Drug and Carrier for various formulations

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ThesoliddispersionofS.D3 batchshowedmaximumdrugcontent[99±0.83] and drug release [98%] and pure drug solution showed the most low release about 20% within 60 minutes, among all the formulationsand this ratio can be used to augment the solubility and dissolution rate of poorlywater soluble drug Ibuprofen. It was observed that the drug release was increasedwith increasingthequantityof Aminated Guar gum.

StabilityStudy

After storage the formulation was analysed for various parameters, resultsare showed in Table7.

Table 7:- StabilitystudyofbestformulationSD3.

Characteristic	Initial	10days	20 days	30 days	45 days
Appearance	White	Nochange	Nochange	Nochange	Nochange
Texture	Smooth	Smooth	Smooth	Smooth	Smooth
Drugcontent(%)	99.80±0.48	99.5±0.45	99.04±0.62	98.89±0.45	98.79±0.54
%of Drugrelease	97.41 ±0.16	97.23±0.34	96.45±0.22	96.24±0.43	96.01±0.42

Allthe values are expressedasmean±SD,n=3

From the table 7, there was no visible changes in the appearance of theformulation SD3and the drug content and dissolution profile of the optimizedformulationwasrelatedto theinitial reference.

Conclusion:-

The purpose of theexisting study was developed solid dispersion of NSAIDS like Ibuprofen by using functionalized guar gum. The results of FTIR study andDSC study confirmed that there is no chemical interaction or no incompatibilityamid the drug and excipients. The solid dispersion technique using Aminated guar gum would be aneffectiveapproachforincreasingthesolubilityandincreasingdissolutionbehaviourofpoorlywatersolubledrug than the native Guargum.

The *in vitro* dissolution study was performed for the prepared formulations. Based on the results SD3 was shown highest drug release99.41%within24hrs.

StabilitystudywasconductedasperICHguidelinesandtheresultsshowedthat there isno physicalor chemical change.

It may be concluded that the Solubility of Drugs Can be improved by using Funtionalized Guar gum in the Solid Dispersion, which provides a wide scope for the therapeutic efficiency.

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RESEARCH ARTICLE

Good Documentation Practices: A Need of Pharmaceutical Industry

Mr. Pravin D. Kolekar*, Prof. Avinash Mahadeo Bhagwat
Department of Pharmacy, Yashoda Technical Campus, Satara, 415015 India.
*Corresponding Author E-mail: **pravin86003@gmail.com**

ABSTRACT:
Good Documentation Practice (GDP) is a term used in the pharmaceutical industry. Good Documentation is an integral part of good manufacturing practices. it is essential for the integrity of data collection and reporting for supporting development, registrations, commercialization, and life-cycle management of pharmaceutical products. Documents are a mirror to show actual image of any pharmaceutical Industry. Such measures that collectively and individually ensure documentation, whether paper or electronic, is attributable, legible, traceable, permanent, contemporaneously recorded, original and accurate.

KEYWORDS: Good Documentation Practice, Pharmaceutical industry, Paper Document, Electronic Document.

INTRODUCTION:

The GDP can be defined as "Good documentation practice is an essential part of the quality assurance and such, related to all aspects of GMP" this definition is based on WHO. Good Documentation Practices (GDP) are methods for recording, correcting and managing data, documents and records, to ensure the reliability and integrity of information and data throughout all aspects of a product's lifecycle. Clearly written documents prevent errors of various activities in pharma each and every activity is written in specific documents such as SOPs and strictly followed. Verbal communications may be the create errors andthere was no proof. So, to minimize error by good document practice that all important documents such as Master formula record, procedure and record must be free from errors and documented.

1. Type of formulation
2. Country requirements
3. Availability of ERP or SAP system.[1]

The U.S. Food and Drug Administration (FDA) Set Some GDP Standards, others fall under the Current Good Manufacturing Practice (CGMP). All pharmaceutical, bioscience andhealthcare companies, as well as their vendor partners, must observe GDP or face warnings or penalties levied by the FDA. According to the World Health Organization (WHO), The purposes of GDP are:

1. To define the specifications and procedures for all materials and methods ofmanufacture and control.
2. To ensure that all personnel concerned with manufacturing know what to do andwhen to do it.
3. To ensure that authorized persons have all the information necessary to decide whether or not to release A batch of A drug For Sale.
4. To ensure the existence of documented evidence, traceability and to provide recordsand an audit trail that will permit investigation.
5. To ensure the availability of the data needed for validation, review and statisticalanalysis.

Documentation is also key to GMP compliance for it ensures traceability of all development, manufacturing, and testing activities. Documentation provides the route for auditors to assess the overall quality of operations within a company and the final product (3). For example, with the FDA Code of Federal Regulations (CFR), 21CFR 211.180(e), records and reports, states: "written records…shall be maintained so that data therein can be used for evaluating…the quality standards of each drug product…". This extract thereby links the importance of good documentation to GMP. This paper presents an overview of good documentation practices applicable to those working in the pharmaceutical and healthcare sectors. Specific topics for discussion include the following:

1. Good manufacturing practice and documentation including errors and error correction
2. Document fundamentals
3. Document creation
4. Document management
5. Types of documents
6. Best practices for document creation, including style and layout
7. Completing documents and record-keeping
8. Electronic records
9. Document storage [2]

Objectives:

1. Establish, control, monitor and record all activities, which directly or indirectly impact on all aspects of the quality of medicinal products.
2. Appropriate good documentation practice should be applied with respect to the typeof document
3. Ensure that the document should maintained accuracy, integrity, availability and legibility during the document life cycle.
4. Document should be free from error and during any point of if error identify then rectify with proper reason for correcting including sign and date.
5. The Term "Written" in any document means recorded/document on media from which data may be rendered in a human readable form.
6. Site Master File: A document describing the GMP related activities of themanufacturer.[3]

Importance of Documentations:

As per GMP documentation control "If it not written down, then it did not happen" The document provides information on when, where, who, why and how to complete the task. The document provides evidence proving that the tasks have been completed as they should be. 4

Scope:

The Good documentation practices are target to both paper as well as electronic data or manually filled records or generated electronically in a GDP environment.[5]

Basics of Documentations:

To meet industry standards, it is critical that all documentation follows GDP when it affects:

1. GMP processes
2. Material or product identity, quality, purity, strength and safety
3. The validated state of GMP product manufacture, facilities, equipment, computer systems and testing methods.

It is recommended that your company has a policy or procedure outlining the expected GDP standards, particularly for those requirements that may be unique to your company – for example, using a specific pen color or when and how to use scanned documents/records as original data. [6]

Purposes:

1. To provide the basic guide for good document practices with regard to creation, approval, review, maintenance, correction or errors, verification and archiving etc.
2. To define the specifications and procedures for all materials and methods ofmanufacture and control.
3. To ensure the existence of documented evidence, traceability and to provide records and an audit trail that will permit investigation.
4. Ensures availability of data for validation, review and statistical analysis.
5. To ensure that all personnel concerned with manufacturing know what to do andwhen to do it.
6. To improve performance.
7. Regulatory requirements. [7]

Key Quality and Regulated Documents:

1. **Concise:**
   Present information clearly so it can be easily understood with no room for misinterpretation. For example, the date format "05/06/12" can cause confusion. Use one that is unambiguous, such as "05 Jun 2012."

2. **Legible:**
   Information should be readable and leave no room for error (for example, hand-written data that are not legible may cloud data analysis or result in "Missing Data").

3. **Accurate:**
   Documentation should be error-free—properly reviewed, verified and approved. Information should be recorded as an event happens and not after the fact, so as to avoid recording "What You Remember" rather than "What Actually Happened."

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4. **Traceable:**
   Documentation should be traceable. make it clear who logged the information, what it was, and when and why it was documented.

Do's and Don'ts with Document:

1. Use black or blue permanent, indelible ink.
2. Make clear, complete and legible entries.
3. Make an entry when an event happens (Not Later).
4. Make corrections that are legible and traceable. for example, when a correction is required, put a line through the error, make the correction next to the error, include an explanation (If it is not self-explanatory), and initial and date the correction.
5. If it is not appropriate to fill in a space in a document (Such as an empty page), enter "N/A," your initials and the date so that no further information can be added later.
6. Follow established standard operating procedures (Sops)—for example, document review and approval processes, version control, and date and time formats, as well as record retention, change control, electronic signature (If applicable) and so on.
7. Provide training to everyone in company.
8. Do not use pencils or erasable ink.
9. Do not use "Write-Out" or any masking devices.
10. Do not make corrections that are not traceable (For example, overwriting entries with no date, initial or explanation).
11. Do not use "Sticky" notes.
12. Do not back-date or post-date.
13. Do not use ditto marks.
14. Do not use asterisks that may cause confusion (Such as using the same asterisk for different footnotes).
15. Do not transcribe data.
16. Do not use unbound laboratory notebooks without page numbers (That is, avoid any doubt concerning missing pages).[8]

Types of Documentations:

Documentation refers to both printed forms and electronic systems. Broadly speaking, documentation types can be classified as:

1. Specifications
2. Manufacturing and packaging instructions
3. Standard operating procedures
4. Records.

More specifically, the various types of documents found within a typical pharmaceutical organization include:

1. Technical agreements
2. Confidentiality agreements
3. Technical reports
4. Quality system related documents
5. Quality Manual
6. SOP's
7. Validation protocols and reports
8. Deviation reports
9. Audit plans
10. Validation master Plans and validation documents including URS, DQ, FAT, IQ, OQ, PQ, and validation reports
11. Test material related documents including product specification, test material receipt,and reports
12. Personnel related documents including training records
13. Facility related documents including floor plans, HVAC plans, and environmentalspecifications
14. Deviation forms including unplanned deviations and system failure investigation
15. Change control
16. Worksheets, notebooks, and logbooks documentation must be clear, free from errors, subject to regular review, and be kept up-to date.[9]

MATERIALS AND METHODS:

Constituents of Good Documentation:

- Approve, review and update documents
- Changes and current revision status of documents identified
- Relevant versions of applicable documents available at points of use
- Documents remain legible and readily identifiable
- Documents of external origin identified, and their distribution controlled
- Prevent unintended use of obsolete documents, and archiving

Principle:

Good documentation constitutes an essential part of the quality assurance system and is key to operating in compliance with GMP requirements. The various types of documents and media used should be fully defined in the manufacturer's Quality Management System. Documentation may exist in a variety of forms, including paper-based, electronic or photographic media. The main objective of the system of documentation utilized must be to establish, control, monitor and record all activities which directly or indirectly impact on all aspects of the quality of medicinal products. The Quality Management System should include sufficient instructional detail to facilitate a common understanding of the requirements, in addition to providing for sufficient recording of the various processes and evaluation of any observations, so that ongoing application of the requirements may be demonstrated. There are two primary types of documentation used to manage and record GMP compliance: instructions (directions, requirements) and

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records/reports. Appropriate good documentation practice should be applied with respect to the type of document. Suitable controls should be implemented to ensure the accuracy, integrity, availability and legibility of documents. Instruction documents should be free from errors and available in writing. The term 'written' means recorded, or documented on media from which data may be rendered in a human readable form. [10]

Documentations Challenges:
Because of the complexities of record keeping in the pharmaceutical industry, there are inherent challenges that companies face in regard to GDP. Some of the most commonconcerns compliance officers must keep in mind include:

1. Lack of proper record-keeping when documents are transferred from one department or facility to another.
2. Critical oversight regarding document issue, data collection and document review.
3. Consistent labeling which includes identification codes, document revision codes, product identification codes and product lot numbers. [11]

Documents Errors:
Common documentation errors that commonly appear in FDA warning letters and reports from other regulatory authorities include**:**

1. Documentation not contemporaneous
2. Use of ditto marks
3. Use of signature stamp
4. Failure to use ink as specified by procedure
5. Incorrect ink used for entries causing illegible data when a substance was spilled
6. Logbook corrections failed to identify person who made the changes
7. Obscured original data
8. Use of pencil
9. Inaccurate records
10. Sample sequence table and audit trail not documented (to draw on the commonly used phrase: "if it is not documented, it didn't happen")
11. Handwritten changes not dated
12. Write-overs, multiple line-through, and use of "white-out" or other masking device. the most common GMP citation occurs with correction of errors when information is recorded. Correction of documentation errors should include:

A. Draw a single line through the error,
B. Make the correction next to the error,
C. Write an explanation for the error,
D. Sign and date the correction.
E. It is recommended that these common errors are highlighted in training on the creation and use of documentation.[12]

Documents Fundamentals:
There are many different types of documents found within pharmaceutical organizations, each serving a different purpose. Although there are different document types, documents can generally be placed into a small number of categories cascading down the quality system. With the types of documents and some of the errors relating to documentation use, it is useful to consider at this point how documents come together and what the basics of a document are. This is illustrated in Figure 1.

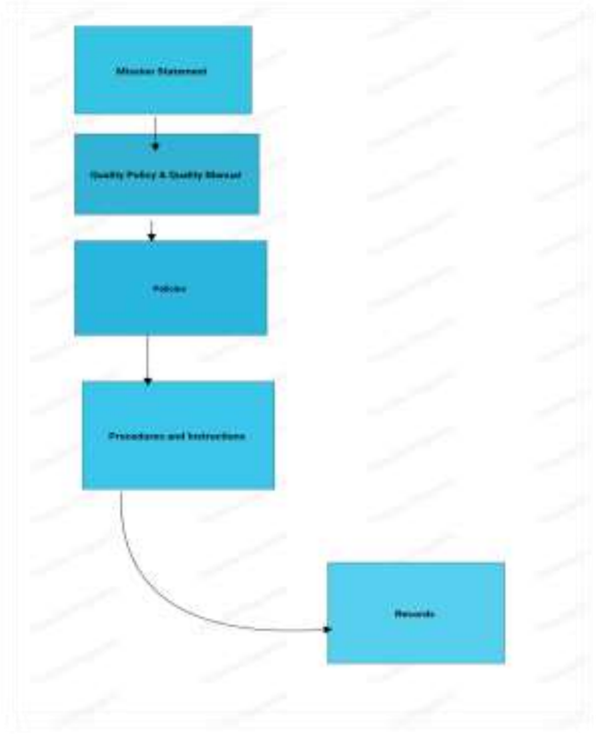

Figure 1. Classic Documentation Hierarchy

The vast majority of documents are procedures or records. The most common examples of a procedure within the pharmaceutical organization are standard operating procedures (SOP). A record is often related to a specific SOP and carries the confirmatory details required of that SOP. For example, the SOP for a sterility test record should require details such as the product name, its batch number, and its test result.

Documents Creation:
The creation of documentation can be conceived very much like a process. In doing so, the first stage can be described as event capture. However, the information or event has no status unless it can be verified or approved, which is the second stage. The last part of the process is to communicate the event, in this context by circulating and implementing the document. To illustrate this, consider a laboratory test common to many microbiology laboratories – the gram stain technique. To

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document the procedure, we need to write down the steps which capture the process. As part of a controlled system, the steps need to be verified as being correct and the procedure "signed of" (approval stage). The procedure can then be issued in into routine use along with associated training, which is the communication stage.

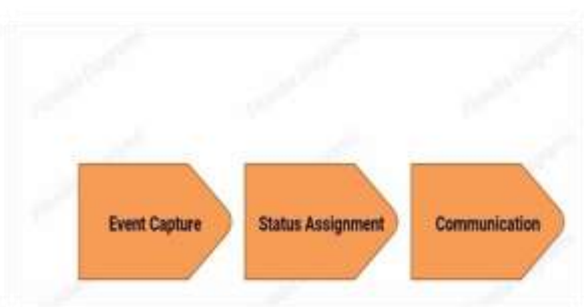

Figure 2. Documentation Flow Path

Documents Control:
Further considerations regarding the system controlling documentation include:
1. Documents should be available at point of use
2. Masters, including electronic versions, are held under control
3. There is control over format
4. There is a system for changes, approval, and re-issue
5. There is control of documents of an external origin.

The majority of these requirements also make up the elements of the "documentation lifecycle"— From document creation, through its use, to its storage and archiving, and then to its eventual retirement and possibly replacement by a revised version. The control of documents necessitates the following steps:

Documents Approval:
Documents must be approved for use. They must be approved, signed, and dated by appropriate authorized personnel.

Handwritten Entries:
1. Adequate space needs to be provided for expected handwritten entries
2. Handwritten entries must be in indelible ink
3. Critical entries must be independently checked (second person verified)
4. No spaces for handwritten entries should be left blank. If unused, they are crossed out or "n/a" (or similar text) entered
5. Ditto marks or continuation lines are not acceptable
6. A stamp in lieu of a handwritten signature is not acceptable.

Documents Copies:
1. Copies need to be clear and legible
2. Errors must not be introduced
3. Documents should be regularly reviewed and kept current,
4. Documents should be retained and readily available for audits
5. Archived documents must be retrievable for the appropriate duration
6. Electronic document management systems must be validated
7. Electronic records must be backed up.

Documents Modification:
1. Handwritten modifications are signed and dated
2. Altered text should not be obscured (e.g., no obliterating the text through crossing-out)
3. Where appropriate, the reason for alteration must be noted (for example, "error." is acommon abbreviated reason, indicating "entry error"),
4. Controls exist to prevent the inadvertent use of superseded documents
5. Electronic versions should only be modified by authorized personnel
6. Access to electronic documents must be controlled by password or other means.

A history (audit trail) must be maintained of changes and deletions to electronic documents. Well-designed documentation and appropriate documentation are paramount. It is necessary to document every aspect of the process, activities, and operations involved with drug and medical device manufacture. If the documentation showing how the product was made and tested (which enables traceability and, in the event of future problems, recall from the market) is not correct and in order, then the product does not meet the required specification and could be considered to be adulterated.[13]

Document Style and Layout:
It is often helpful to adopt a specific document style for consistency of operations. Elements of the style should be specified in an approved procedure. These might include (9):
- Logo
- Pagination/ layout to prevent confusion and ensure the document is kept in order
- Headers and footers
- Font including the size is useful to minimize errors introduced when changingbetween fonts
- Page numbers
- Executive summary
- Changes - Change control is important for traceability
- Circulation list - to ensure the document is reviewed

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and received by the appropriatepersonnel
- Table of contents
- Authorization levels stated on document
- Cross references
- Revision history
- Definitions
- Content (context and meaning)
- A clear area for recording problems/ incidents
- Use of pictures, flow charts, diagrams as suitable alternatives to text.

Care should be taken in designing and stylizing documentation. Documents must have unambiguous contents. The title, nature, and purpose should be clearly stated. They must be laid out in an orderly fashion. Documents must be easy to check. Reproduced documentsmust be clear and legible. Many people do not consider the importance of how key information is presented within a document. This can result in the reader wasting time or not conducting the correct tasks. For example, a poorly structured document could ask the user to conduct a task that requires some action to be performed prior to the subject task – but only mentions the prework at the end of the document and with no reference to the pre-work at the beginning. Helpful considerations for layouts include the following:
- Cover page with identifiers and status
- Table of contents – creating a road map through the document
- Scope and applicability section
- Introduction
- Information and instructions in a logical sequence
- Additional information and detail.

Numerical information must include the correct use of units. The use of color coding in graphical information, such as black lines for existing pipework and red lines for new pipework, might also be useful. The narrative must consider writing style, nomenclature, and dealing with errors and corrections It is useful to consider different styles to accommodate the different reading styles of readers. There are thought to be three styles based on the linguistic, logical and spatial talents. These can be summarized as (10):
- Linguistic talent. There is a strong ability to write and talk fluently. Phrases like “gift of the gab” often apply. Individuals in this category can also write and read well. Shakespeare had linguistic talent.
- Logical talent. There is strong ability to think logically and are quick in calculating odds and statistics. Albert Einstein had logic talent.
- Spatial talent. There is strong ability to image things in the “mind’s eye.” These people often have good navigational skills such as Christopher Columbus. The following should be considered whenever possible:
- Narrative – written text
- Tabular – tables
- Charts – bar charts, pie charts
- Color – bold – underlined text
- Pictures – photographs, images
- Diagrams – 2d,3d
- Process logic – flowcharts

Including various combinations of the above is extremely useful. A flow chart might help with navigating the document while pictures are very useful in dressing procedures for entry into cleanrooms.[14]

Completing Documents and Record Keeping:
After documents have been designed, prepared, and approved, they must be used and completed properly. For example, where documents require the entry of data, these entries must be made in clear legible handwriting using a suitable indelible medium -- not a pencil. Sufficient space must be provided for entries. With such entries, it is important that any correction made to a document or record must be signed or initialed and dated; the correction must permit the reading of the original information. Where appropriate, the reason for the correction must be recorded. With record-keeping in general, a record must be kept at the time each action is taken. All activities concerning the conduct of preclinical studies, clinical trials, and the manufacture and control of products must be traceable.[15]

Documents Storage:
Storage of critical records must at secure place, with access limited to authorized persons. In relation to this, 21CFR 211.180(d) states "…these records or copies…shall be subject to photocopying or other means of reproduction as part of such inspection. Records that can be immediately retrieved from another location by computer or other electronic means shall be considered as meeting the requirements of this paragraph."

The storage location of document must ensure adequate protection from loss, destruction, or falsification, and from damage due to fire, water, and other disasters. Records which are critical to regulatory compliance or to support essential business activities must be duplicated on paper, microfilm, or electronically, and stored in a separate, secure location in a separate building from the originals. If electronic, photographic or other data processing systems are used for the retention of documents, an appropriate storage for required duration is necessary to protect against loss or damage. It is particularly important that during the period ofretention, the data can be rendered retrievable and legible within an appropriate period of time. This means having a

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validated system of data recall. The data should also be availablein a legible form. Rapid retrieval of reports and data is essential for audits.[16]

CONCLUSION:

From the above review we are concluded that good documentation practices are a need of pharmaceutical industry. documentation is essential to achieve total approach towards GMP. The goal of good documentation practices is defined manufactures of system information. Documentation also serves as existence of evidence and allows traceability.

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RESEARCH ARTICLE

CAPA: An important concept of Quality Assurance in Pharmaceutical Industry

Chavan Pooja Ajit[1]*, Avinash Mahadeo Bhagwat[1], Atul Prabhakar Chaudhari[2]
[1]YSPM's Yashoda Technical Campus, Faculty of Pharmacy (B. Pharm), Satara
[2]Lecturet Smt SS Patil Institute of Pharmacy, Chopda, Dist. Jalgaon 425107
*Corresponding Author E-mail: **chavanp978@gmail.com**

ABSTRACT:
CAPA is used in the improvements to be made in product, process or quality system to eliminate non-conformities and other undesirable situation. CAPA could be regulatory concept that focuses on systematic investigations to search out the root cause, understanding and correcting discrepancies while attempting to avoid their reoccurrence. Instructions for a way they must be handled within the organization just in case of potential product problems, customer complaints or action to eliminate the cause of a detected nonconformities or incident. Regulatory inspections give more importance for CAPA, for the explanation, it will high light the systems followed within the company additionally because the technical capability of the people concerned. Changes proposed are to be verified and validated to confirm the effectiveness and quality attribution. CAPA is also an integrated part of ISO: 13485 and Good Manufacturing Practice (GMP) for medical products. The FDA defines the purpose of a CAPA procedure as: collecting and analyzing information, identifying and investigating product and quality problems, and taking appropriate and effective corrective and/or preventive action to prevent their recurrence. There is not a regulatory defined framework for the CAPA process only different requirements. The CAPA system investigation document will provide a clear picture of how the standard system works and hence, Regulatory Inspectors give lot of importance to audit this method. Real root cause is to be identified with scientific proof and which further may not be generated. This review provides comprehensive views on steps involved in corrective action and preventive action (CAPA), mechanism of taking CAPA enabling to boost the system of quality management. CAPA is a component of the overall quality management system. As per the FDA documents CAPA accounts for 30-50% of FDA-483 forms issued for noncompliance.

KEYWORDS: Non-conformities, Root Cause, Regulatory Inspectors, Quality Management System.

INTRODUCTION:

Troubleshooting problems and attempting to search out and to avoid potential problem is atypical activity for many companies. Ability to correct existing problems or apply controls to avoid potential problems is vital for customer satisfaction and efficient business practice. Hence, the primitive person during this process is commonly sufficient documentation actions. Appropriate documented actions gives essential historical data for never-ending quality improvements plan are important for any product that has got to meet regulatory guideline demanded by FDA & ISO and other quality system. This

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can be the rationale to implement a politician Corrective Action/ Preventive Action (CAPA)program.[1] Corrective and Preventive Action (CAPA) is recognition of potential problems of non-conformance/ undesirable affecting the standard of research and to get rid of them to avoid recurrences. It's set of action that laws or regulations need a corporation to require in manufacturing, documentation, procedure, or systems to analyses and to get rid of recurring non-conformance. Non- conformance is to seek out after the systematic evaluation and examine the basis reason for non-conformance. Non-conformance could also be customer complaint or market complaint or a top quality management system, or false of machinery or misinterpretation of written instructions which to be implement work. It must be systematically applied and recognize its ability to get rid of further recurrence of non-conformation. The Eight disciplines problem solving method or 8D framework are often used for an efficient method of structuring of CAPA. CAPA is concept with the subsequent Good Manufacturing Practice (GMP), Hazard Analysis and Risk-based Preventive Controls (HACCP/HARPC) and various ISO business standards. Its main aim to specialize in systematic analysis of the foundation reason behind identified problems or identified risks in a shot to avoid their recurrence (i.e. corrective action) or to avoid occurrence (i.e. preventive action).Corrective action to search out by Statistical Process Control (SPC) which is implement to response to customer compliant, unacceptable levels of product non-conformance, issues to search out during an indoor audit, moreover as adverse or unstable trends in product and process monitoring. Preventive actions are implementing to response to the identification of potential sources of non-conformity. To certify that corrective and preventive action is effective, the systematic inspection of the basis causes of failure is pivotal. CAPA is an element of the general Quality Management System (QMS).[2]

Definition:

Correction:

An action that's taken to eliminate the non-conformity or undesirable situations. However, correction doesn't address causes.

Corrective Action:

A corrective action is term as encompasses the method of reacting to product problems, customer complaints or other non-conformities and fixing them. CA means to forestall the recurrence of non-conformities or undesirable situations. To eliminate the issues that was previously present and reduces their chances of occurring again.

Preventive Actions:

A preventive action may be a process for detecting potential problems or non-conformances and eliminating them. PA means to stop the occurrence of non-conformities or undesirable situations. It is answerable for identifying and keeping away the problem that will arise within the future.[3]

Difference between Corrective and Preventive Actions [4]

Corrective Action	Preventive Action
Reactive	Proactive
Initiated from customer complaint	Initiated from customer suggestion
Existing non-conformity	Opportunity for improvement
The issues is currently affecting the process	No immediate issue affecting the process
A solution is requires now (at least a temporary one)	No need to do something right away
Changes must be made	Changes might not be implemented
A root-cause analysis is required	A root-cause analysis is not required

Objective:

The purpose of the corrective and preventive action subsystem is to gather and analyze information, identify and investigate product and quality problems, and take appropriate and effective corrective and/or preventive action to avoid their recurrence.

Verifying or validating corrective and preventive actions, communicating corrective and preventive action activities to responsible people, providing relevant information for management review and documenting these activities are important in dealing effectively with product and preventing their recurrence, quality problems and minimizing device failures. One amongst the foremost important quality system elements is that the corrective and preventive action subsystem.[5]

What is CAPA per ICH Q10?

A structured approach to the examination process should be used with the target of determining the root cause. The level of effort, formality and documentation of the examination should be equivalent with the extent of risk, in line with ICH Q9.

As per ISO 9000:2005 corrective actions is taken to prevent recurrence whereas preventive action is taken to prevent occurrence.

Corrective and preventive actions even have introduced within the quality management process as defined in the Project Management Book of Knowledge (PMBOK). For understanding regular business of corrective and preventive action is additionally considered a tool within Six Sigma operations. CAPA has strong parallels with Design for Six Sigma (DFSS), won't to design new products or redesign existing products.[6]

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ICH Q10 Recommends a Product Lifecycle Approach:[6]

Pharmaceutical Development	Technology Transfer	Commercial Manufacturing	Product Discontinuation
The product or process variability is explored. Methodology of CAPA is beneficial where corrective actions and preventive actions are incorporated into the iterative design and development process.	CAPA are often used as a good system for feedback, feed forward, and continual improvement.	CAPA should be used, and therefore the effectiveness of the actions should be evaluated	CAPA should continue after the merchandise is discontinued. The impact on product remaining on the market should be considered, moreover other products that may be affected

Why CAPA?

Corrective Action and Preventive Action or that may be termed as corrective action is to enhance imposed on an organizational process to eliminate undesirable situations and non-conformity. Corrective and preventive action is beneficial to abolish the matter occurred during the manufacturing process. This helps to avoid the re-occurrence of the matter during manufacturing and analysis.

- The estimate the effectiveness of a given action plan.
- The CAPA also certify that information associated with nonconforming products and quality problems emanate from those to blame for the prevention of such problems or assuring the standard of such products.
- The corrective action and preventive action is accountable with for information collection, analysis, identification and analysis of quality and merchandise problems and taking effective and suitable preventive and/or corrective actions in an a trial to stop their occurrence or re-occurrence.
- Providing necessary information for management review, and documenting the activities that are vital in handling with quality and merchandise problems, preventing problem re-occurrence and minimizing or preventing device failure.
- CAPA is a necessary tool for improving organizations processes.
- CAPA is chargeable for validating and verifying preventive and corrective actions, communicating preventive action and corrective action activities to responsible people.[7]

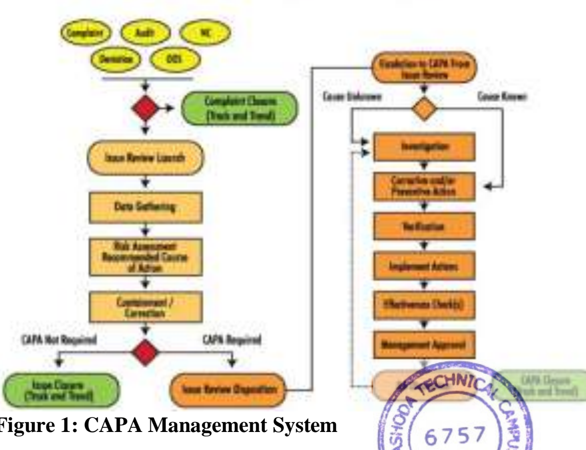

Figure 1: CAPA Management System

5. Fundamental of CAPA Quality Process:

CAPA management software is intended to clarify FDA requirements for corrective/preventative action processes.

To provide effective safeguards versus regulatory risk, CAPA is mostly a module within a comprehensive quality management system. If not, it's usually capable of integrating with management systems for audits, nonconformities, document management, change control, and other capabilities.

Unfortunately, the FDA offers minimal auspice on selecting a CAPA system. At a high level, the CAPA system must:

- Include CAPA procedures which address quality system requirements
- Facilitate data investigate to spot the sources of product quality concerns
- Enable the organizations to watch trends for preventive action
- Integrate with surrounding systems and QA processes to assure the info quality
- Facilitate statistical analysis and formal failure investigations
- Allow organizations to validate the success of preventive or corrective actions

These requirements definitely don't translate on to software features. As such, an intimate understanding of the CAPA quality process is required.

1. Detection:

The problem identification and CAPA detection phase requires a suitable documentation of the problem at hand. The outline should be complete including who, what, when, where, why, and the way many.

Moreover, a risk assay should be performed supported compliance risk. The results of the chance analysis should acquaint the CAPA timeline. It should be obvious that low-risk issues don't have the identical sense of urgency as high-risk issues.

2. Investigation and Root Cause Determination:

The quality management teams should confide to rapid investigation and root cause determination. There are several methods for conducting analysis including:

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- Brainstorming
- Flowcharting
- Fishbone Diagrams
- Affinity Diagrams
- Physics of Failure

Typically, root cause determination is aid. by quality management systems. With end-to-end traceability, you can easily track every change and action from starting to end with fully integrated, closed-loop quality processes.

3. Proposed Corrections:
In this next phase, correction and condition should be completed as soon as possible to forestall further disruption. Moreover, organizations should proactively review processes and procedures to spot broader issues. Just in case of a product-related issue, field correction and/or recall are also required.

4. Implementation:
At this time, long-term corrective and preventative actions work to see or eliminate the explanation for nonconformity. A corrective action is an action that deletes the cause of nonconformity. On the flipside, a preventative action is an action to delete the explanation for potential nonconformity.

5. Verification of Effectiveness:
Finally, validate or verify corrective and preventative action effectiveness. Once a CAPA investigation is complete, determine if nonconformities are determined. Moreover, to see if corrective and preventative actions haven't created new areas of inconsistencies. Any changes done to the assembly process that were made to deal with a difficulty should even be seen as a replacement source of potential problems. [8]

Stages of CAPA

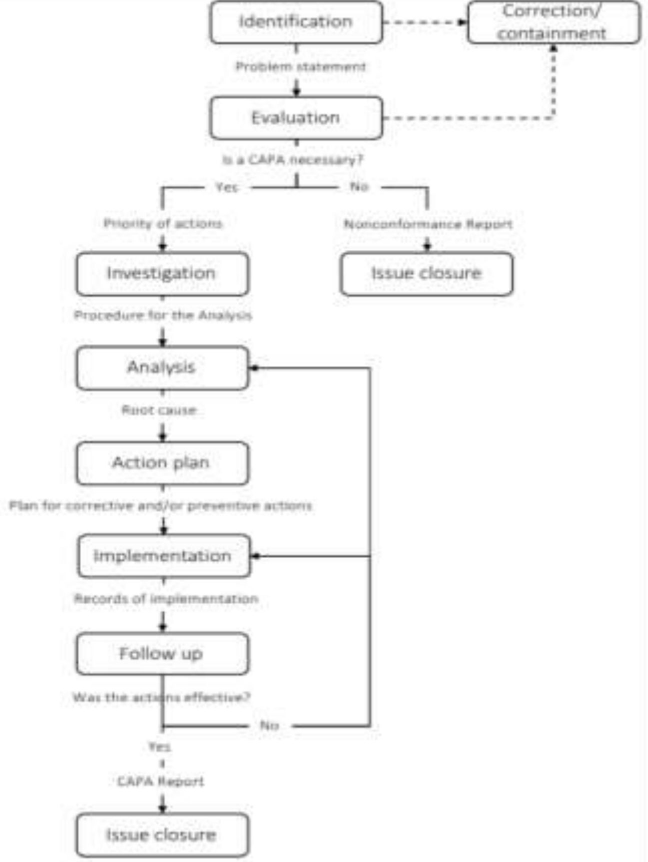

Figure 2: Flow Chart of Stages

1. Identification:
Identification defines an existing problem or a possible problem. It includes:
- Explanation of problems
- Documentation of accessible evidence
- Internal audits, process monitoring, data trends, customer complaints and QA inspection

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2. **Evaluation:**

The objective is to see the necessity for action and also the level of action required

- Potential impact – concerns the impact on company and clients in terms of cost, product quality, safety and customer satisfaction
- Risk – level of risk related to the matter
- Remedial action – potential impact and risk assessment to assist decide the desired remedial action

3. **Investigation:**

- Review various parameters associated with a controversy e.g. equipment, materials, procedures, analyst training, software capabilities and environmental parameters
- Fix individual responsibilities and resources requirement such as finances, manpower and equipment. These requirements are worked out and documented

4. **Analysis:**

Analysis leads to the root cause of the problem

- Collect data and try to list out the possible sources so as to arrive at the root cause. Data may come from such sources as records, processes, service information, operations, etc.
- Root cause will not simply deal with symptoms but help uproot the main contributing factors

5. **Action Plan:**

- Action plan is aimed at correcting and preventing future occurrence of failure. Plan involve to changes to be made in existing procedures and assignment of responsibilities
- All modifications and changes must be tell too concerned personnel, departments and suppliers
- Employee training is an essential part of any change and is always part of an action plan

6. **Implementation:**

Implementation stage involves:

- Execution of identified tasks
- Modification in documents
- Modification in processes
- Modification in environmental conditions
- Provision of training on modifications

All stages in implementation stage need to be correctly documented

7. **Follow Up:**

The follow up confirms completion of the identified tasks and also assesses the appropriateness and Effectiveness of the action taken. The validatory report must record:

- If the main cause of the problem is solved
- Any resulting secondary situations have been corrected
- Proper controls established to prevent future recurrence
- Actions taken that have no other adverse effects
- Adequate monitoring arrangements that are in place which assigned the responsibilities

A CAPA report should be signed by the authorized or well experience personnel within the operational divisions of the organization. [9]

What is a CAPA Report?

A CAPA (Corrective and Preventive Action) Report may be a tool utilized in identifying, addressing, and preventing regulatory and organizational non-conformance. Compliance officers record the main points of a problem or incident on a CAPA Report form, which primarily includes a summary of the event, date of occurrence, items and other people involved, corrective actions taken, and preventive action established to avoid future recurrence.

Here is an example scenario of a CAPA report's response to an incident:

Sample Incident:

A working man is injured because of the improper use of commercial machinery.

Corrective Action – this is the action taken to instantly address the prevailing problem.

The worker is given trending and brought to the closes hospital for further treatment.

Preventive Action:

This is often the action taken to stop recurrence of the identical problem.

Workers are trained on the right use of commercial machinery to avoid recurring accidents and injuries.

When to put in writing CAPA Report:

A CAPA report is flexible and may be used for various sorts of issues and incidents. However, not every event warrants a CAPA report. Quality teams must utilize risk management techniques to see the severity of an event and choose if a CAPA report is required.

Here are some situations where a CAPA report is beneficial:

- If the problem is severe/urgent

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- If the difficulty is recurring
- If the difficulty is systemic[10]

CONCLUSION:

Corrective action and Preventive action (CAPA) is very important path towards improvement and effectiveness of Quality Management System in pharmaceutical industry. It plays a vital role in Quality Risk Management System. The root cause analysis of any problem or deviation is often easily done by implementing CAPA. Pharmaceuticals, healthcare and medical devices industries should strictly follow the CAPA system to maintain the consistent quality in their products. CAPAs, is performed to help an organization to improve its competitive position by improving processes. Corrective and Preventive Actions are integral parts of a continuous improvement program. An effective CAPA system can help to prevent a warning letter during an audit. CAPA is used as tool for forward and backward traceability. CAPA compliance with the requirements, and get correct nonconformity and which are reasonable to measure the limit of risk. Fortunately, when pharmaceuticals products are failed then we demand for investigation to identify why it's occurred. CAPA is a process which investigates and solves problems, identifies causes, take corrective action and preventive recurrences of the root causes. The ultimate purpose of CAPA is to assure the problem that can never be experienced again. Hence, it is compulsory to implement the CAPA system in pharmaceutical industry to maintain the consistent quality in their products.

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RESEARCH ARTICLE

RP-HPLC Method Development and Validation of Tadalafil in Tablet Dosage form

Chavan Pooja Ajit[1], Shelar Reshma Dattatraya[1], Shelake Pallavi Ramchandra[1], Avinash Mahadeo Bhagwat[1], Dr. Ajit Bhiva Ekal[2]
[1]YSPM's Yashoda Technical Campus, Faculty of Pharmacy (B. Pharm), Satara
[2]Managing Director of Insta Vision Laboratory and Service, Satara
*Corresponding Author E-mail: **chavanp978@mail.com, reshmadshelar10@gmail, pallavishelakestr@gmail**

ABSTRACT:
Tadalafil is a phosphodiesterase 5 inhibitor accustomed to treat dysfunction, benign prostatic hyperplasia, and pulmonary arterial hypertension. Tadalafil is an orally administered drug and it's marketed worldwide under the name Cialis. Analytical method development and validation play important roles within the discovery development and manufacture of pharmaceuticals. These methods is to make sure the identity, purity, potency, and performance of drug product. The bulk of the analytical development effort goes into validating a stability indicating HPLC–method. Analytical method development could be a process of proving that the developed chromatography method is suitable for its intended use within the development and manufacturing of the pharmaceutical drug substance and drug product. All analytical methods that are intended to be used for analyzing any clinical samples will have to be validated. The target of the strategy validation is to demonstrate that the strategy is suitable for its intended purpose because it is stated in ICH guidelines.

KEYWORDS: Tadalafil, Phosphodiesterase 5 Inhibitor, Erectile dysfunction, High performance liquid chromatography, Validation.

INTRODUCTION:
Male erectile dysfunction has been defined as the persistent inability to attain and maintain an erection adequate to permit satisfactory sexual performance. Although erectile dysfunction is regarded as a benign disorder, it has a medical and social impact due to its high prevalence, costs and implications for the quality of life for many men and their partners.

A recent review concludes that the prevalence of erectile dysfunction of all degrees is 52% in men 40 to 70 years old, with the incidence increasing with advancing age. Normal erectile function requires the coordination of psychological, hormonal, neurological, vascular and anatomic factors. Alteration of any of these factors is sufficient to cause erectile dysfunction.[1]

Tadalafil is reversible phosphodiesterase type 5 (PDE5) inhibitor approved for the treatment of erectile dysfunction (ED). As a category PDE5 inhibitors (including sildenafil and vardenafil), enhance erectile response to sexual stimulation by increasing penile blood flow. The duration of action of Tadalafil is longer than sildenafil or vardenafil.[2]

Tadalafil (CIALIS) is an orally administered PDE5 inhibitor has been developed for a treatment for erectile dysfunction. When sexual stimulation causes the local release of nitric oxide gas, which plays a central role within the vasodilation of erectile tissues by stimulating guanylyl cyclase activity, consequently raising intracellular concentrations of cyclic guanosine monophosphate (cGMP) and relaxing vascular smooth muscle. This leads in smooth muscle relaxation and inflow of blood into the penile tissues, thereby producing an erection. Thus tadalafil is indicated for the treatment of male erectile dysfunction. Tadalafil has no effect within the absence of sexual stimuli.[1]

Tadalafil is a phosphodiesterase 5 inhibitor accustomed treats erectile dysfunction, benign prostatic hyperplasia, and pulmonary arterial hypertension. Tadalafil is practically insoluble in water. It does not possess any ionisable groups within the pH range of 1-11 and, subsequently, doesn't demonstrate any changes in solubility in aqueous buffers in this range. It is freely soluble only in solvents like as dimethylsulfoxide and dimethylformamide.[1]

This molecule has 2 chiral centers and thus four different stereoisomers are also found. The molecule obtained in the process described is within the RR form. Crystallization studies show that Tadalafil doesn't exhibit polymorphism.[1]

Chemistry:

1. Synonyms: Adcirca, Cialis, GF196960, HSDB7370, IC351, ICOS351, Tadalafil, Tadalafil Lilly, UNII-742SXX0ICT
2. IUPUC:(6R,12aR)-6-(1,3-benzodioxol-5-yl)-2-methyl-2,3,6,7,12,12a-hexahydropyrazino[1',2':1,6]pyrido[3,4-b]indole-1,4-dione
3. Formula: C22H19N3O4
4. Molar mass: 389.411 g·mol−1 [3]

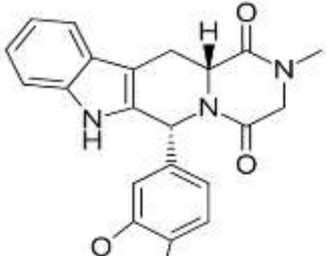

Figure 1: Chemical Structure of Tadalafil

This study is to develop an easy and accurate RP-HPLC method for the estimation of Tadalafil in tablet dosage form. The method validation is to demonstrate that the strategy is suitable for its intended purpose because it is stated in ICH guidelines. The strategy was validated for linearity, precision, accuracy, specificity, and limit of detection, limit of quantification, robustness and system suitability.[4]

MATERIAL AND METHOD:

Material:

Tadalafil pure drug sample obtain from Tadacip 20 tablet which is manufactured at Cipla Ltd, India. HPLC grade Acetonitrile was procured from E. Merck Ltd, India. SQ grade Potassium Hydrogen Orthophosphate was purchased from Fisher Scientific. AR grade Orthophosphoric Acid was purchased from Rankem. Milli-Q water was used throughout the experiment. Tadacip 20 tablets were purchased from local pharmacy.

Instrumentation:

Analysis was performed on a Summit HPLC chromatographic system, Low Pressure Quaternary Gradient Dionex manufacturer equipped with ASI-100 Automated sample injector, LPG-4 HPLC Pump, programmable variable wavelength PDA-3000 detector. Chromatographic separation was achieved by Agilent eclipse C_{18} column (4.6 x 250mm, 5um). The HPLC system was equipped with "Chromeleon 6.8 SR 11" software to acquire and process the data. Peak purity was checked the PDA detector.[5]

METHODOLOGY:

Standard solutions:

Weigh accurately and transfer about 40mg Tadalafil standard in 200ml volumetric flask. Add about 150ml of Mobile phase. Sonicate for 5 minutes. Allow the solution to attend room temperature and dilute up to mark with mobile phase.

Mobile phase:

Phosphate Buffer pH 4.0: 1.360gm. of Potassium Di-hydrogen Orthophosphate dissolved and diluted in 1000 ml water. Adjust the pH to 4.0 with dilute ortho phosphoric acid. Phosphate buffer pH 4.0: Acetonitrile (50:50). Mix, Sonicate and filter through 0.45 micron nylon filter paper.

Chromatographic Conditions:

An isocratic condition HPLC analysis was performed an Agilent Eclips C18 (150 x 4.6mm, 5um) maintained at conditions (30°C). Chromatographic separation was achieved with mobile phase and mixture of at flow rate of 1.0ml/min and injection volume of 20µl and also the run time is 6 min. the Tadacip 20 was scanned under conditions and from the spectra maxima of 284nm was observed.

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Assay of formulation:
Procedure:
Weigh 20 Tablet at a time. Calculate Average weight. Crush Mix to make uniform and perform assay from it.

Sample Preparation:
Weigh and transfer sample powder containing about 40 mg of Tadalafil in 200ml volumetric flask. Add about 150ml of mobile phase. Sonicate for 20 minutes. Allow to attend room temperature. Dilute up to mark with mobile phase. Filter with whatman 41 or equivalent filter paper.

Inject blank, standard and Sample preparation as sequence given below,

(1) Blank (2) Standard preparation (5 replicates) (3) Blank (4) Sample preparation (5) Standard preparation (Bracketing standard)

Perform calculation for system suitability parameters –% RSD of retention time and area of standard replicates, cumulative %RSD of retention time and area of replicate standards with bracketing standard.

System suitability criteria:
1. %RSD for retention time of replicates of standard preparation should not be more than 1.0%
2. %RSD for area of replicates of standard preparation should not be more than 2.0%
3. Theoretical plates for all the standard injections should not be less than 2000. Report Theoretical plate of first replicate of standard preparation.
4. Tailing factor for all the standard injections should not be less than 2.0. Report tailing factor of first replicate of standard preparation.
5. Cumulative %RSD for retention time of replicates of standard preparation with retention time of bracketing standard should not be more than 1.0 %
6. Cumulative %RSD for area of replicates of standard preparation with area of bracketing standard should not be more than 2.0%

If system suitability found within limit, perform calculations for content of Tadalafil Tablet by using formula, Calculate Content of Tadalafil (mg/Tablet) for sample preparations independently by using formula described below.

(Area of Tadalafil in sample preparation) W1 (mg) 200 P1
= --**x---x-----x-x**W3 (gm.)
(Avg. area of Tadalafil Standard preparation) 200 W2 (g) 100

Where,
W1 = Weight of Tadalafil standard taken for preparation of standard preparation (in mg)
W2 = Weight of Tadalafil sample taken for preparation of sample preparation (in g)
W3 = Average weight of Tablet in gm.
W = Weight of sample in gm.
P1 = % Purity of Tadalafil Standard

Acceptance Criteria:
Each film coated tablet content:
Tadalafil per tablet = 18.00mg to 22.00mg

% Assay = 90.00% to 110.00%

Method validation:
A. Specificity and system suitability:
- Experimental design:
1. Inject mobile phase to observe interference at interested peaks.
2. Prepare and inject std preparation individually (as per final conc. Of mixed std preparation).

- **Acceptance criteria:**
1. There should not be interference of any peak at interested peaks.
2. System suitability parameter should meet and assay for tadalafil 20mg should be within limit.

B. Precision
a) Repeatability:
- Experimental design:
  Perform assay in six individual replicates of sample. Perform % RSD of assay.
- Acceptance criteria:
  % RSD for assays should be within less than 2%

b) Intermediate precision:
- Experimental design:
1. Perform assay in six individual replicates of sample by other person on other day with other set of chemicals.
2. Perform %RSD of six sample preparations.
3. Also calculate % variation of average assay values obtained via repeatability and intermediate precision.

- **Acceptance criteria:**
1. Assay for all samples should be within limit.
2. % RSD for assays for intermediate preparation should be less than 2%.
3. % variation of average assay values obtained via repeatability and intermediate precision should be less than 3%.

C. Accuracy:
- **Experimental design:**
1. Prepare samples in triplicate by taking weight equivalent to 80%, 100% and 120% of working levels and perform analysis.
2. Calculate % recovery by factors amount added vs.

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amount recovered.
3. Calculate %RSD of recovery at each level for triplicate preparations.

- **Acceptance criteria:**
1. % recoveries of individual preparation should be 98 to 102%.
2. % RSD at each level should not be more than 3%

D. Linearity and range:
- Experimental design:
1. Prepare standard preparations at each 80%, 100% and 120% of working level and inject them.
2. Determine co-relation coefficient by plotting linearity graph. Calculate % y intercept

- **Acceptance criteria:**
1. Graph should be linear and co-relation coefficient should be not less than 0.999
2. % y intercept should be within ±2%

E. LOD AND LOQ
- **Experimental design:**
1. 5ppm solution to be prepared and signal to noise ratio is determined.
2. LOD is determined by preparing diluted solutions with signal to noise ratio about 3.
3. Level of concentration at which peak got detected repeatedly but not necessary to be precise is LOD.
4. LOQ is determined by preparing diluted solutions with signal to noise ratio above 3 and about 10.
5. Level of concentration at which peak got detected with precise area (%RSD NMT 2%) is LOQ.

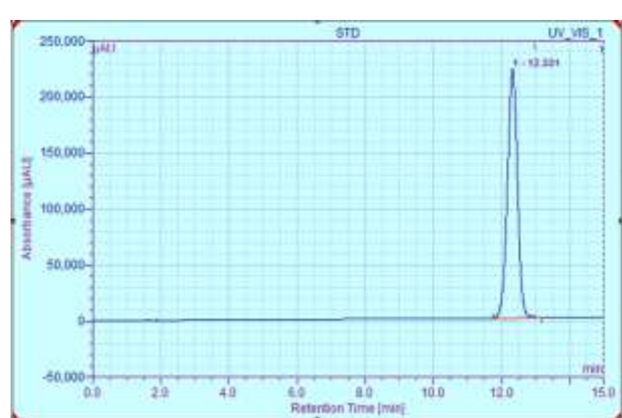
- **Acceptance criteria:**
1. For LOD Single to noise ratio is about 3.
2. For LOQ Single to noise ratio is about 10.
3. For LOQ, %RSD for replicate injections should be less than 2 %

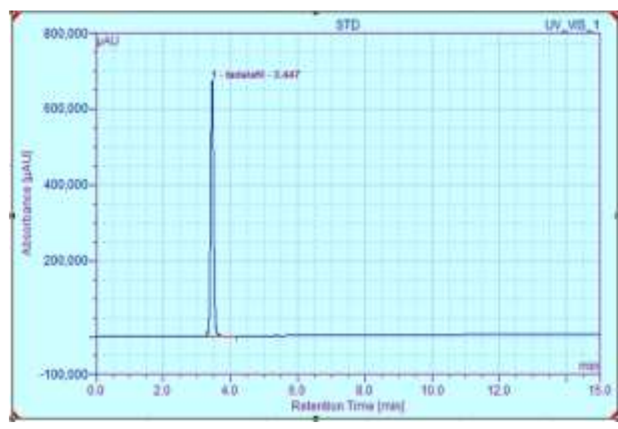
F. Robustness:
- Experimental design:
1. Perform analysis of sample by changing flow rate as 0.8 and 1.2ml per minutes.
2. Perform analysis of sample by changing wavelength 282 nm and 286nm.
3. Calculate % cumulative RSD of assay obtained at repeatability and by changing parameters.
- Acceptance criteria:
1. % cumulative RSD of assay obtained at repeatability and by changing parameters should not be more than 3 %

Non-conformance:
Specify the non-conformance (if any) observed during method validation.

RESULT AND DISCUSSION:
Method development and optimization:
In order to optimize the LC conditions for the estimation of Tadalafil in tablet the following trials were performed. Initially a mobile phase consisting of 50mM Potassium Di-hydrogen Orthophosphate (pH 5.0): Acetonitrile (80:20 %v/v) at a flow rate of 1.0mL/min was used on an Agilent Eclipse C18 column (250 x 4.6) column at ambient temperature using mobile phase as diluent, Tadalafil did not elute under these conditions,

In the next trial, same column was employed but the mobile phase was changed to mobile phase consisting of 10 mM Potassium Di-hydrogen Orthophosphate (pH 5.0): Acetonitrile (70:30% v/v) at a flow rate of 1.0 mL/min was used on an Agilent Eclipse C18 column (250 x 4.6) column at ambient temperature.





Tadalafil eluate with at 12.331 with theoretical plates 4234 (limit NLT 2000 and Tailing factor 1.58 (NMT2) which well within the limit but as retention time is more and will time consuming during analysis therefore focusing on reduction of retention time drastically. pH of buffer solution reduced with dilute Orthophosphoric acid

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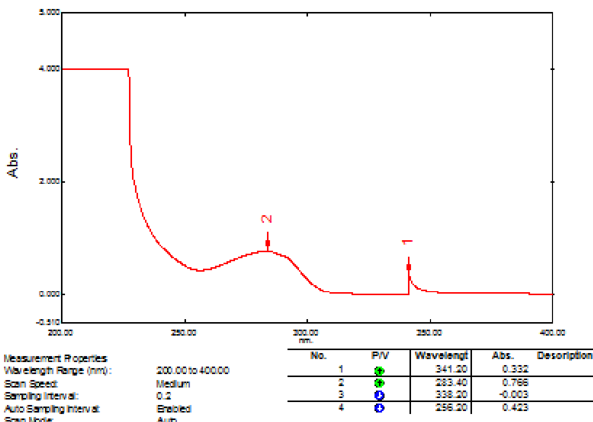
solution. Organic Solvent content in mobile phase composition increased and also temperature of column increased.

Tadalafil eluate with at 3.447 with theoretical plates 3431 (limit NLT 2000 and Tailing factor 1.55 (NMT 2) which well within the limit. Thus further injection of same std is done and to conclude precision. Corresponding area, RT and system suitability parameters observed.

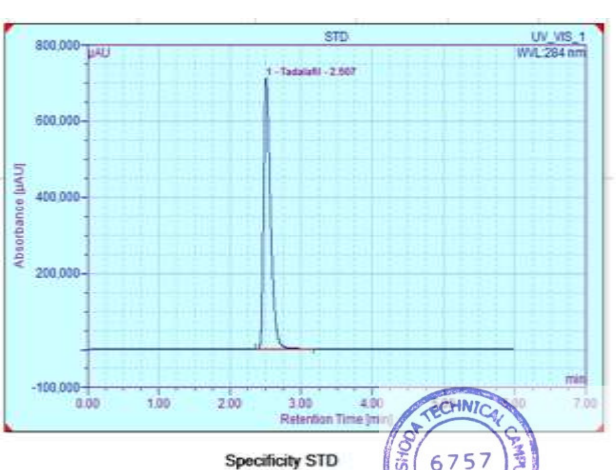
Lambda max is determined before conducting next trial and found 284nm.Next trial conducted with mobile phase consisting of 10mM Potassium Di-hydrogen Orthophosphate (pH 4.0): Acetonitrile (50:50% v/v) at a flow rate of 1.0mL/min was used on an Agilent Eclipse C18 column (250 x 4.6) column at 30°C.



Method validation

A. Specificity And System Suitability:

Specificity demonstrated by observing interference of mobile phase (Diluent). System suitability parameters (% RSD of area, Retention time, Theoretical Plates and Tailing factor) demonstrated by injecting standard preparation in replicate.



Specificity STD

Table no1: Specificity and System Suitability

Std Inj No	Retention Time	Area	Theoretical Plates	Tailing Factor
1	2.507	80482.079	3436	1.59
2	2.507	81838.312	3359	1.59
3	2.508	80682.928	3412	1.58
5	2.507	80378.131	3398	1.57
5	2.509	80181.986	3422	1.58
% RSD	0.04 (Limit NMT 1 %)	0.81 (Limit NMT 2 %)	Limit: NLT 2000	Limit: NMT 2

No interference observed from Mobile phase (Diluent).

Conclusion – Method found specific and capable to achieve System suitability.

B. PRECISION:

A. Repeatability:

The repeatability was demonstrated by preparing the standard solution at 200ppm concentration and six independent consecutive sample preparations at 200 ppm. System suitability found within limit. Relative standard deviation of assay value for six preparations found within 2 %.

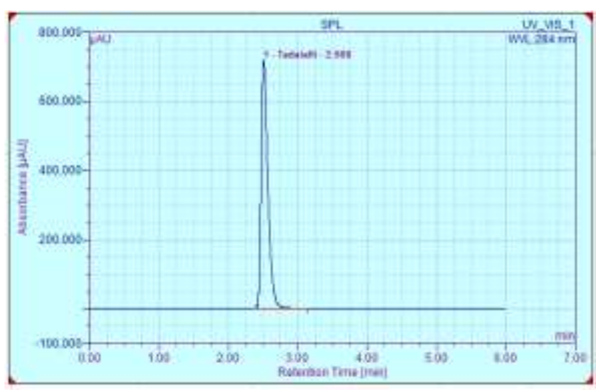


Table no 2: Repeatability Suitability System

Std Inj No	Retention Time	Area	Theoretical Plates	Tailing Factor
1	2.507	80550.869	3436	1.59
2	2.508	80234.452	3392	1.58
3	2.507	81223.21	3414	1.57
4	2.505	80877.456	3433	1.58
5	2.508	80281.714	3390	1.58
% RSD	0.05 (Limit NMT 1 %)	0.52 (Limit NMT 2 %)	Limit: NLT 2000	Limit: NMT 2

Average std area = 80633.5402

Sample	RT	Area	% Assay
1	2.508	81050.398	101.35
2	2.507	81124.947	101.16
3	2.507	81038.768	101.38
4	2.508	81098.332	100.71
5	2.509	81168.295	101.06
6	2.509	81068.679	100.84
% RSD (NMT 2 %)			**0.27**

B. Intermediate Preparation:

The Intermediate Precision was demonstrated by preparing the standard solution at 200ppm concentration

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and six independent consecutive sample preparations at 200ppm. By other person on other day with other set of chemicals. System suitability found within limit. Relative standard deviation of assay value for six preparations found within 2%. % variation of average assay values obtained via repeatability and intermediate precision found within 3%

Table no 3: Intermediate Preparation System Suitability

Std Inj No	Retention Time	Area	Theoretical Plates	Tailing Factor
1	2.507	84508.997	3369	1.58
2	2.507	84122.505	3278	1.57
3	2.504	83453.121	3245	1.56
5	2.505	84467.887	3314	1.56
5	2.504	83567.886	3243	1.57
% RSD	0.06 (Limit NMT 1 %)	0.59 (Limit NMT 2 %)	Limit: NLT 2000	Limit: NMT 2

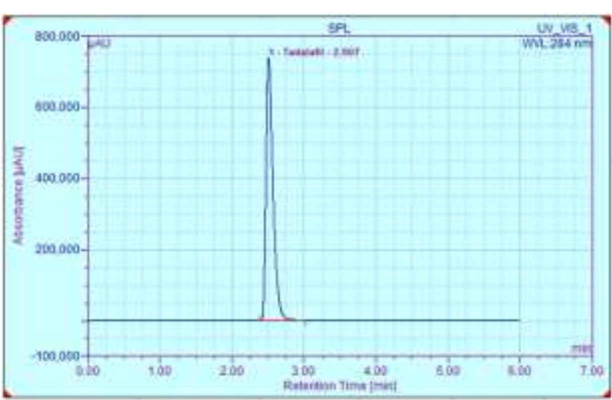
Average std area = 84024.0792

Sample	RT	Area	% Assay
1	2.507	84235.965	100.47
2	2.507	84156.235	100.52
3	2.509	84578.691	100.92
4	2.509	84269.523	101.28
5	2.508	84638.419	100.84
6	2.507	84398.651	100.86
% RSD (NMT 2 %)			**0.29**
% RSD with repeatability (NMT 3 %)			**0.30**

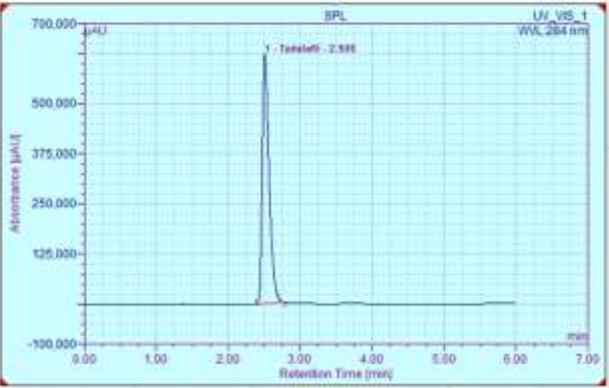
Conclusion – Method found Precise.

C. ACCURACY:

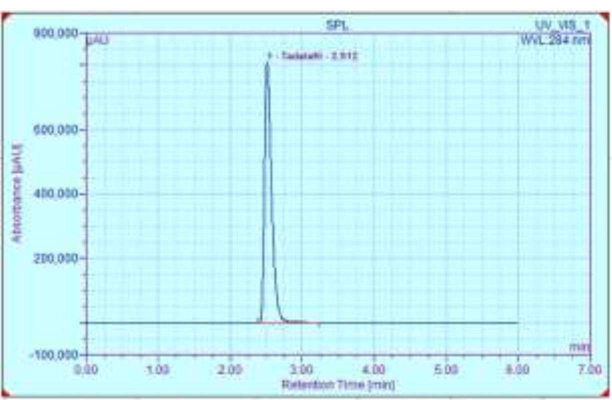
To determine the accuracy of the method, recovery studies were carried out in triplicate by using different concentrations of pure drug in the pre analyzed samples with 3 different concentrations of sample that consists of 80 %, 100 % and 120 % of the pure drug. The accuracy was expressed as the percentage analytes recovered.



Accuracy SPL 100 %



Accuracy SPL 80 %



Accuracy SPL 120 %

Table no 4: Accuracy

Std Inj No	Retention Time	Area	Theoretical Plates	Tailing Factor
1	2.506	84122.127	3312	1.55
2	2.505	84613.560	3214	1.55
3	2.504	83948.654	3344	1.54
4	2.508	84026.334	3370	1.56
5	2.509	84386.329	3351	1.55
% RSD	0.08 (Limit NMT 1 %)	0.33 (Limit NMT 2 %)	Limit: NLT 2000	Limit: NMT 2

Sample	% Recovery	Average % recovery	% RSD
80 %	99.66	99.54	0.36
80 %	99.13		
80 %	99.82		
100 %	98.94	99.39	0.41
100 %	99.73		
100 %	99.51		
120 %	99.15	99.25	0.55
120 %	98.76		
120 %	99.84		
Limit		**Limit 98 to 102 %**	**NMT 3 %**

Conclusion – Method found Accurate.

D. LINEARITY AND RANGE:

From the standard stock solution, the various dilutions of Tadalafil in the concentration of 160.0, 200.0, 240.0 ppm three level standard solutions of each were prepared. The solutions were injected using 20 µL injection volumes in

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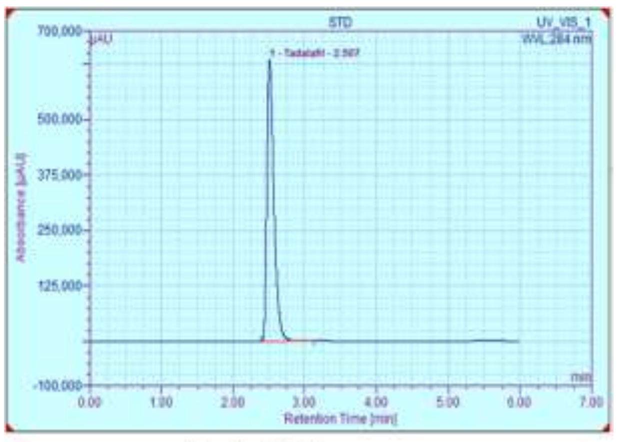
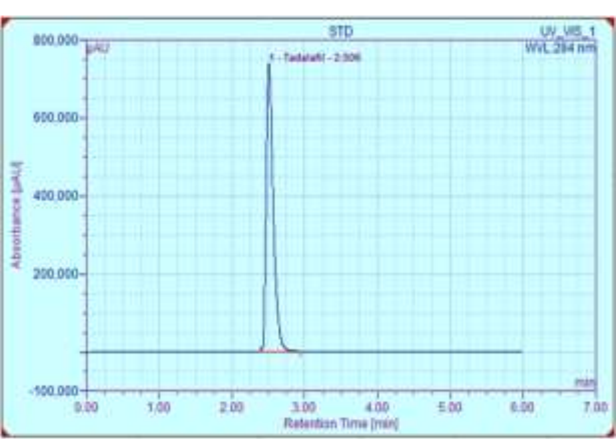
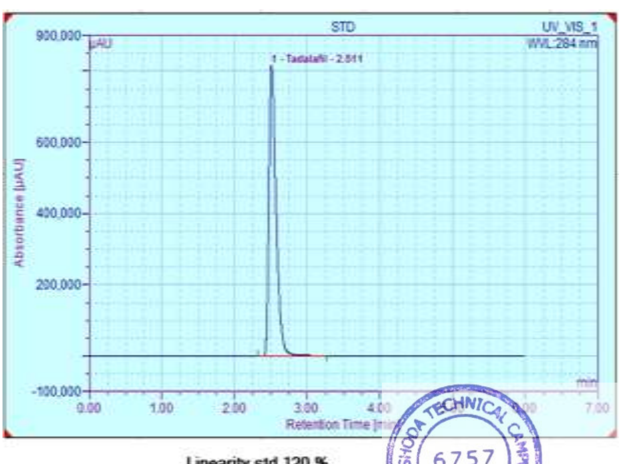
to the chromatographic system at the flow rate of 1.0 ml/min and the effluents were monitored at 284nm, chromatograms were recorded. Calibration curve of Tadalafil was obtained by plotting the peak area ratio versus the applied concentrations of Tadalafil by using average of each sample. The linear correlation coefficient (R2) was found to be 1.000 and %y intercept is 0.0035%



Linearity std 80 %



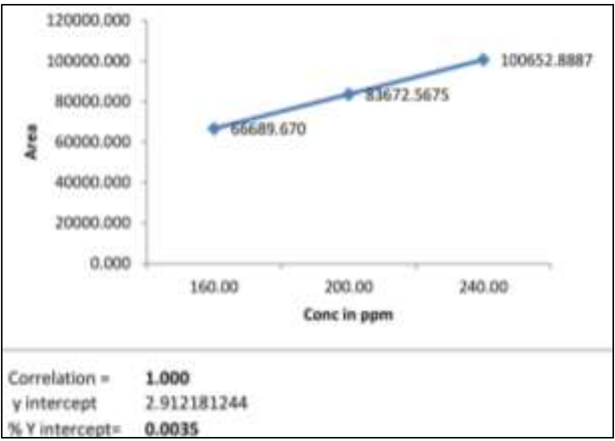
Linearity std 100 %



Linearity std 120 %

Table No 5: Linearity and Range

Sr. No.	Conc. ppm	Area	Average
1	160.0	66710.597	66689.670
2	160.0	66638.432	
3	160.0	66719.981	
4	200.00	83676.153	83672.5675
5	200.00	83698.982	
6	200.00	83642.568	
7	240.00	100407.985	100652.8887
8	240.00	100598.458	
9	240.00	100952.223	



Conclusion – Method found Linear in the range 80 % to 120 % of working level

E. Limit of Detection and Limit of Quantitation (LOD and LOQ):

The limit of detection and limit of quantification means the lowest concentration of analytes in the sample are detected and quantified. LOD and LOQ was found as listed below

Table no 6: LOD and LOQ

Parameter	Obtained value
LOD	0.00035 ppm
LOQ	0.00523 ppm

F. ROBUSTNESS:

Robustness of the method was determined by intentionally changing some operating conditions such as flow rate and wavelength. The flow rate as per the developed method is 1.0ml/min. It has been purposely changed to 0.8ml/min and 1.2ml/min and the chromatogram was developed as well as the wavelength of developed method is 284nm. It has been purposely changed to 282nm and 286nm and the chromatogram was developed.

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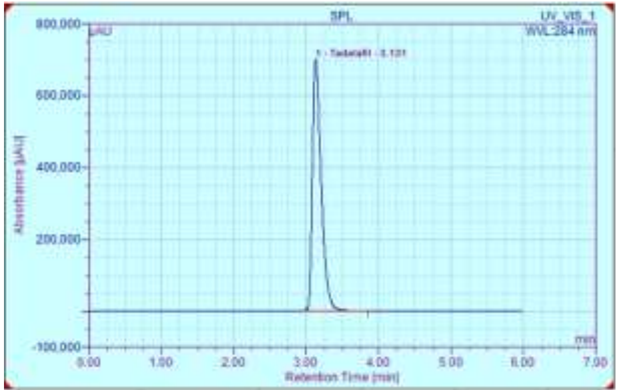
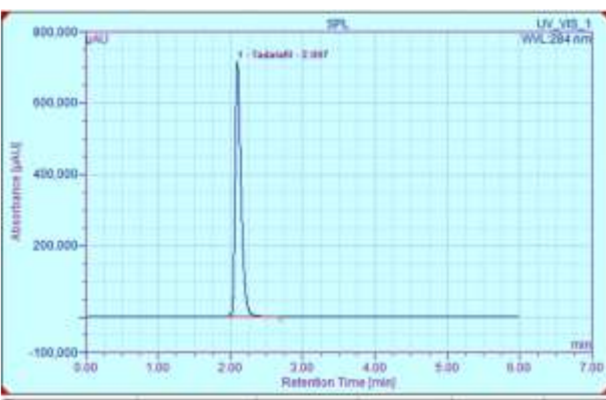
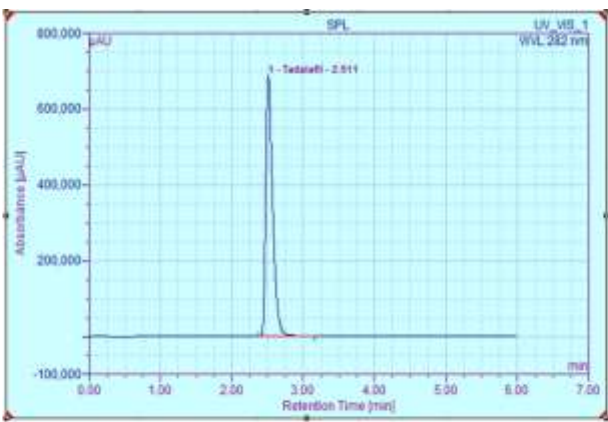


SPL 0.8 ml / min



SPl 1.2 ml / min



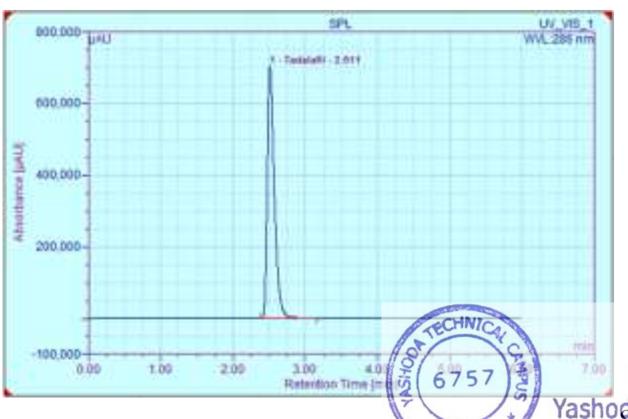


Table No 7: Robustness system suitability flow rate = 0.8 ml

Std Inj No	Retention Time	Area	Theoretical Plates	Tailing Factor
1	3.128	100735.480	3388	1.59
2	3.126	100123.563	3321	1.58
3	3.124	99131.118	3339	1.57
4	3.125	99790.264	3350	1.59
5	3.128	99520.003	3297	1.59
% RSD	0.06 (Limit NMT 1 %)	0.61 (Limit NMT 2 %)	Limit: NLT 2000	Limit: NMT 2

Parameter	Condition	RT	Area	% Assay	% Cumulative RSD with Repeatability
Change in flow rate	0.8 ml /min	3.131	99300.247	100.11	0.38

Table no 8: Robustness system suitability flow rate = 1.2 ml /min

Std Inj No	Retention Time	Area	Theoretical Plates	Tailing Factor
1	2.098	68319.994	3335	1.56
2	2.097	67222.965	3334	1.55
3	2.098	68210.003	3330	1.54
5	2.099	68427.675	3323	1.56
5	2.096	69276.998	3245	1.56
% RSD	0.05 (Limit NMT 1 %)	1.07 (Limit NMT 2 %)	Limit: NLT 2000	Limit: NMT 2

Parameter	Condition	RT	Area	% Assay	% Cumulative RSD with Repeatability
Change in flow rate	1.2 ml /min	2.097	68411.243	101.43	0.24

Table no 9: Robustness system suitability detection wavelength = 282 nm

Std Inj No	Retention Time	Area	Theoretical Plates	Tailing Factor
1	2.097	80130.345	3287	1.58
2	2.096	80023.776	3186	1.56
3	2.093	80211.543	3233	1.57
5	2.095	81235.867	3295	1.58
5	2.097	80425.971	3231	1.58
% RSD	0.08 (Limit NMT 1 %)	0.61 (Limit NMT 2 %)	Limit: NLT 2000	Limit: NMT 2

Parameter	Condition	RT	Area	% Assay	% Cumulative RSD with Repeatability
Change in wavelength	282nm	2.096	77812.828	100.11	0.38

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Table no 10: Robustness system suitability detection wavelength = 286 nm

Std Inj No	Retention Time	Area	Theoretical Plates	Tailing Factor
1	2.097	79145.885	3212	1.56
2	2.095	78230.870	3198	1.55
3	2.092	79431.114	3243	1.54
5	2.094	79786.240	3230	1.57
5	2.096	79555.476	3211	1.55
% RSD	0.09 (Limit NMT 1 %)	0.76 (Limit NMT 2 %)	Limit: NLT 2000	Limit: NMT 2

Parameter	Condition	RT	Area	% Assay	% Cumulative RSD with Repeatability
Change in wavelength	286nm	2.097	80160.9116	100.78	0.23

% Cumulative RSD of % assay observed for changing parameters calculated and found within limit i.e. below 3 %.

Conclusion – Method found Robust.

Non-conformance: Specify the non-conformance (if any) observed during method validation.

CONCLUSION:

The proposed RP-HPLC method was simple, sensitive, precise and accurate for determination of Tadalafil n tablet dosage form. The results obtained for all validated parameters were within the limits; hence the proposed method can be easily applied for the quantification of Tadalafil in routine quality control pharmaceutical laboratories. The analytical method used for determination of assay of Tadalafil Tablet is within acceptance criteria for the analytical parameters such as Specificity and system suitability, Linearity and Range, Precision, Accuracy and Robustness. Hence method stands validated.

ACKNOWLEDGEMENT:

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Role of Aminated derivatives of Natural Gum in Release Modulating Matrix Systems of Losartan Potassium: Optimization of Formulation using Box-Behnken Design

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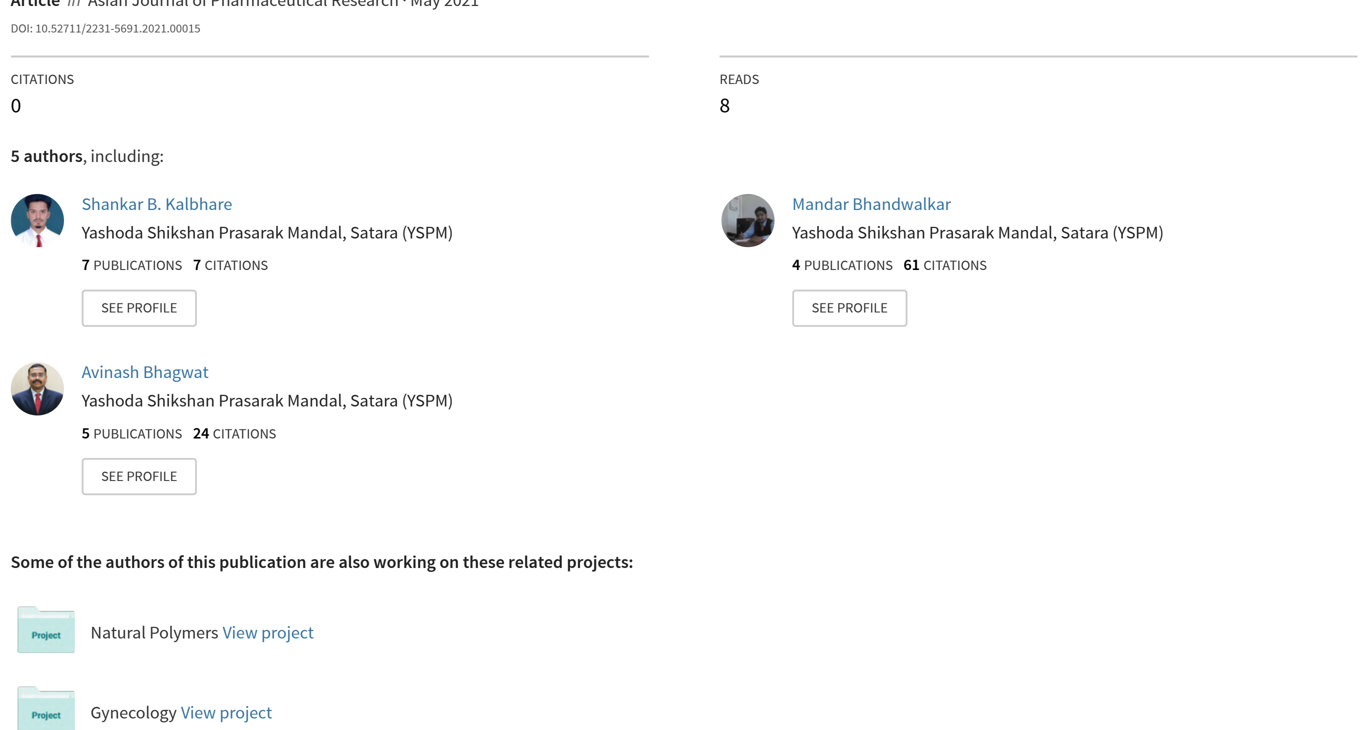

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RESEARCH ARTICLE

Role of Aminated derivatives of Natural Gum in Release Modulating Matrix Systems of Losartan Potassium: Optimization of Formulation using Box-Behnken Design

Shankar B. Kalbhare[1]*, Dr. Vivek Kumar Redasani[2], Mandar J. Bhandwalkar[3], Rohit K. Pawar[3], Avinash M. Bhagwat[1]

[1]Assistant Professor, YSPM's Yashoda Technical Campus, Satara (M.S.) India.
[2]Director, YSPM's Yashoda Technical Campus, Satara (M.S.) India.
[3]Assistant Professor, Late Narayandas Bhawandas Chhabada Institute of Pharmacy, Satara (M.S.) India.
*Corresponding Author E-mail: **kirankal786@gmail.com**

ABSTRACT:

The aim of the present research work was to systemically device a model of factors that would yield an optimized release modulating dosage form of an anti-hypertensive agent, losartan potassium, using response surface methodology by employing a 3-factor, 3-level Box-Behnken statistical design. Independent variables studied were the amount of the release retardant polymers – aminated fenugreek gum *(X_1)*, aminated tamarind gum *(X_2)* and aminated xanthan gum *(X_3)*. The dependent variables were the burst release in 15 min *(Y_1)*, cumulative percentage release of drug after 60 min *(Y_2)* and hardness *(Y_3)* of the tablets with constraints on the Y_2 = 31–35%. Statistical validity of the polynomials was established. In vitro release and swelling studies were carried out for the optimized formulation and the data were fitted to kinetic equations. The polynomial mathematical relationship obtained $Y_2=32.91-2.29X_1-5.68X_2-0.97X_3+0.20X_1X_3-0.005X_2X_3-0.92X_1^2-1.89X_2^2$ explained the main and quadratic effects, and the interactions of factors influencing the drug release from matrix tablets. The adjusted (0.9842) and predicted values (0.9600) of r^2 for Y_2 were in close agreement. Validation of the optimization study indicated high degree of prognostic ability of response surface methodology. The Box-Behnken experimental design facilitated the formulation and optimization of release modulating matrix systems of losartan potassium.

KEYWORDS: Release modulating matrix tablets; Amination of natural polymers; Losartan potassium; Box Behnken statistical design; Response surface methodology.

INTRODUCTION:

Oral drug delivery is the most preferred and appropriate preference as the oral route provides maximum active surface area amongst all drug delivery system for administration of a various drugs. Significance of these dosage forms is due to awareness to toxicity and ineffectiveness of drugs when administered by oral conventional method in the form of tablets and capsules. Developing oral sustained release matrix tablets for drug with constant release rate has always been a challenge to the pharmaceutical technologist [1]. Usually conventional dosage form produces wide range of

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variation in drug concentration in the bloodstream and tissues with consequential undesirable toxicity and poor efficiency, poor bioavailability. So the maintenance of concentration of drug in plasma within therapeutic index is very significant for effective treatment and high bioavailability [2],[3]. Drug release through various matrix system is determined by Water penetration, Polymer swelling, Drug dissolution, Drug diffusion, Matrix erosion have been utilized as formulation sustained release drug delivery. Sustained release dosage forms offer better control of plasma level less dosage frequency, less side effect, increased efficacy and constant delivery [4].

A polymer is a large molecule (macromolecules) composed of repeating structural units. These subunits are typically connected by covalent chemical bonds. Both synthetic and natural polymers are available but the use of natural polymers tier pharmaceutical applications is attractive because they are economical, readily available and non-toxic. They are capable of chemical modifications, potentially biodegradable and with few exceptions, also biocompatible [5]. Derivatization of native polymer led to enhancement in bioadhesive and drug release characteristics [6]. Recently, chemical modification or derivatization of natural polysaccharides has been reported to improve the functional properties of native gums. Reports in the literature suggest that the derivatives of polysaccharides (amine, thiol, carboxymethyl) can be employed to manipulate swelling, bioadhesion and drug release [7]. A few examples of polysaccharide derivatives already reported in literature include aminated fenugreek gum [6], aminated tamarind kernel polysaccharide [8] and aminated xanthan gum [9]. Polymeric material have fulfilled different roles such as binders, matrix formers or drug release modifiers, film coating formers, thickeners, viscosity enhancers, stabilizers, disintegrants, Solubilisers, emulsifiers, suspending agents, gelling agents and bioadhesive.

Losartan potassium (LP) is a potent, highly specific angiotensin II type 1 (AT1) receptor antagonist with antihypertensive activity [10], [11]. It is readily absorbed from the gastrointestinal tract with oral bioavailability of about 33% and a plasma elimination half-life ranging from 1.5 to 2.5 h. Administration of LP in a controlled release dosage form with dual release characteristics i.e., burst release followed by an extended release over 8 h, would be more desirable as these characteristics would allow a rapid onset followed by protracted anti-hypertensive effects by maintaining the plasma concentrations of the drug well above the therapeutic concentration [12].

Response surface methodology (RSM) is one of the popular methods in the development and optimization of drug delivery systems [13]. Based on the principles of design of experiments (DOE), the methodology involves the use of various types of experimental designs, generation of polynomial mathematical relationships and mapping of the response over the experimental domain to select the optimum formulation [14]. Central composite design (CCD), 3-level factorial design, Box Behnken design and D-optimal design are the different types of RSM designs available for statistical optimization of the formulations [13]. Box-Behnken statistical design is one type of RSM design that is an independent, rotatable or nearly rotatable, quadratic design having the treatment combinations at the midpoints of the edges of the process space and at the center. Additionally, it requires fewer experimental runs and less time a and optimized thus provides a far more effective and cost-effective technique than the conventional processes of formulating and optimization of dosage forms [15].

The current study aimed at developing and optimizing an oral release modulating matrix tablet of LP using computer aided optimization technique i.e. Box Behnken statistical design with constraints on cumulative percentage release of drug after 60 min (31–35%). The Independent variables for the present study were: amount of release retardant polymers – aminated fenugreek gum (X_1), aminated tamarind gum (X_2) and aminated xanthan gum (X_3). The dependent variables studied were the burst release in 15 min (Y_1), cumulative percentage release of drug after 60 min (Y_2) and hardness of the tablets (Y_3).

MATERIAL AND METHODS:

Materials:

Losartan potassium was provided as a gift sample by Viraj Pharmaceutical. (Mumbai, India). Carbopol, magnesium stearate and microcrystalline cellulose were supplied by Thermosil Fine Chem industry. (Charhol). Feenugreek gum, tamarind gum and xanthan gum were purchased from Phyto Lifesciences Pvt Ltd. (Gandhinagar, Gujarat). Starch, isopropyl alcohol and ethylene diamine as a supplied by SD. Fine Chemicals limited. (Mumbai, India). Sodium borohydride was purchased from Karan enterprise. (Mumbai, India). All other reagent and solvents used were of analytical grade and used as received.

Amination of natural polymers:

In 3000ml water add 60gm of natural gum. To this solution add aminating agent ethylene diamine (25ml) with continuous stirring at constant temperature (20-60°C) for 6 hr. Then slowly add reducing agent sodium borohydride ($NaBH_4$) for 2 hr until formation of thick gel. Wash this gel several times with ethyl alcohol and

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collect the precipitate of aminated derivative [6], [8], [9]. Synthesized aminated polymer was studied under further parameter for determination of flow properties, chemical stability and thermal properties.

Preparation of compressed matrix:

Drug, carbopol (binder) and the MCC (diluent) were sifted through #40 manually and mixed well to ensure the uniformity of premix blend [16]. Several drug-diluents premixes were then mixed with the selected combination and ratio of hydrophilic polymers (Aminated FG, Aminated TG and Aminated XG), previously sifted through #40, for 5 min. Premix blend was wet granulated with isopropyl alcohol and the granules were sized through #18 and were dried at 45°C for 15 min. Dried LP granules were lubricated with starch and magnesium sterate. The tablets were compressed at an average compression weight of 250mg by cold compression technique on dialed hydraulic press (KBR press) at 12.0mm, circular, flat punches at compressional pressure of 5 tons with 15 s dwell time [17],[18].

Different formulations of Losartan potassium 100mg release modulating matrix tablets were prepared using the following excipients: AFG (7.5-22.5mg), ATG (10–30mg), AXG (12.5–37.5mg), carbopol (30mg), starch (10.25mg), magnesium sterate (1.75mg) and MCC (q.s. to 250mg).

Experimental design:

Box-Behnken statistical screening design was used to optimize and evaluate main effects, interaction effects and quadratic effects of the formulation ingredients on the in vitro release of LP sustained release formulations [17],[19]. A 3-factor, 3-level design used is suitable for exploring quadratic response surfaces and constructing second order polynomial models with Design Expert® (Version 12.0.1.0, Stat-Ease Inc., Minneapolis, MN). This cubic design is characterized by set of points lying at the midpoint of each edge of a multidimensional cube and center point replicates (n = 3). The nonlinear computer-generated quadratic model is given as,

$$\mathbf{Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^{\,2} + b_{22}X_2^2 + b_{33}X_3^2}$$

Where Y is the measured response associated with each factor level combination; b_0 is an intercept; b_1 to b_{33} are regression coefficients computed from the observed experimental values of Y; and X_1, X_2 andX_3 are the coded levels of independent variables. The terms X_1 X_2 and $X^{\,2}$ $(i = 1, 2 \text{ or } 3)$ represent the interaction and quadratic terms, respectively. The selected dependent and independent variables are shown (Table 1, left column) along with their low, medium and high levels, which were selected based on the results from preliminary experimentation [20]. The amounts of Aminated FG (X_1), Aminated TG (X_2) and Aminated XG (X_3) used to prepare each of the 15 formulations are given (Table 2).

Table 1: Variables in Box Behnken design

Factor	Level used, actual (coded)		
	Low (-1)	**Medium (0)**	**High (+1)**
X1=AFG (%)	3	6	9
X2=ATG (%)	4	8	12
X3= AXG (%)	5	10	15
Dependent variables	Constraints		
Y1 = % Burst release in 15 min	10 ≤ Y1 ≤ 15		
Y2 = % Dissolution after 60 min	31 ≤ Y2 ≤ 35		
Y3 = Hardness (kg/cm2)	Maximize (range 3.5–5.5)		
*All percentages were calculated with respect to total tablet weight of 250mg			

Tablet assay and physical evaluation:

The tablets were assayed for drug content using methanol as the extracting solvent, and the sample were analyzed spectrophotometrically (Shimadzu- UV-1800, Japan) at 215 nm [17], [20]. Tablets were also evaluated for the hardness (n = 6) (Monsanto hardness tester), friability (n = 6) (Roche Friabilator, 100 rpm), weight variation (n = 20) and thickness (n = 10) (Vernier caliper).

Table 2: Observed responses in Box Behnken design for losartan potassium release modulating matrix tablet

Batch	Dependent Variables			Independent variables		
	X1 (%)	**X2 (%)**	**X3 (%)**	**Y1 (%)**	**Y2 (%)**	**Y3 (kg/cm^3)**
1	3	4	10	11.87	37.53	3.5
2	9	4	10	9.63	34.27	4
3	3	12	10	7.16	26.76	5
4	9	12	10	6.81	21.84	5.5
5	3	8	5	10.45	35.63	4.5
6	9	8	5	8.56	30.12	4
7	3	8	15	9.27	33.41	3.5
8	9	8	15	8.13	28.73	5.5
9	6	4	5	12.41	37.61	3.5
10	6	12	5	7.23	26.48	5.5
11	6	4	15	10.71	35.54	3.5
12	6	12	15	6.69	24.39	5.5
13	6	8	10	8.87	33.74	5
14	6	8	10	8.13	32.21	4
15	6	8	10	7.33	32.79	4.5

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In vitro drug release studies:
Dissolution studies were performed using the USP II, paddle-rotating method (Electrolab dissolution tester, Electro lab, India) at 37°C ± 0.5°C and 75rpm using 0.1 N HCl (2 hr) and phosphate buffered solution, pH 6.8 (PBS) (10 hr), as the dissolution media. Dissolution studies were carried out in triplicate, maintaining the sink conditions for all the formulations. A 5ml aliquot of sample was withdrawn at regular time intervals, filtered and assayed spectrophotometrically at 205.3nm. The cumulative % drug release was calculated for the formulations [21],[22].

Swelling and erosion studies:
Swelling and erosion studies of the matrix tablets were carried out under conditions identical to those described for the dissolution testing. After 2 hr in 0.1 N HCl and 6 hr in phosphate buffer, pH 6.8, the tablets were removed, gently wiped with a tissue paper to remove surface water and Scanning Electron Microscopy (SEM) study of the hydrated swollen tablets was carried out [17],[23]. Water uptake and mass loss were determined gravimetrically according to the following equations;

Degree of swelling (water uptake)=
(Wet weight - Original dry weight)/(Original dry weight)

Erosion (% mass loss)=
(Original weight - Remaining dry weight)/(Original weight)

Thermal properties:
Differential scanning calorimetry (DSC) experiments were performed on drug, excipients and the optimized formulation using DSC (Perkin–Elmer, Norwalk, CT). The instrument was calibrated using indium standards. Accurately weighed samples (5–10mg) were hermetically sealed in flat bottom aluminum pans and heated from 48 to 300°C at a rate of 10°C per min under an atmosphere of nitrogen. Thermograms were normalized and rescaled as needed before overlapping [11],[24].

Fourier transforms infrared spectroscopy (FTIR):
FTIR studies were performed on drug, excipients and the optimized formulation using Shimadzu FTIR (Shimadzu Corp., India). Background spectrum was collected before running each sample. The samples were analyzed between wavenumbers 4000 and 400 cm^{-1}.

Optimization data analysis and validation of optimization model:
Statistical validation of the polynomial equation generated by Design Expert® was established on the basis of ANOVA provision in the software. A total of 15 runs with triplicate center points were generated. The models were evaluated in terms of statistically significant coefficients, standardized main effects (SME) and R^2 values. Various feasibility and grid searches were conducted to find the compositions of optimized formulation. Various 3-D response surface graphs were provided by the Design Expert software. By intensive grid search performed over the whole experimental region, nine optimum checkpoints formulations were selected to validate the chosen experimental domain and polynomial equations. The optimized checkpoint formulations were prepared and evaluated for various response properties. The resultant experimental values of the responses were quantitatively compared with that of the predicted values. Also, linear regression plots between actual and predicted values of the responses were produced using MS-Excel.

RESULTS AND DISCUSSION:

Characterizations of derivetized natural polymers:
The synthesis polymers are characterized using ATR-FTIR, DSC and XRD studies. In this study, synthesis polymers are confirmed by ATR-FTIR study. The ATR-FTIR study of aminated fenugreek gum, aminated tamarind gum and aminated xanthan gum is confirmed by the appearance of a new peak at 3271 cm^{-1}, 1639.49 cm^{-1} and 2899.01 cm^{-1} respectively corresponding to NH_2 group.

Aminated fenugreek gum:
The DSC thermograms of AFG, in that the AFG shows the broad endothermic peak at 9.94°C with heat of fusion 38.33 J/g, and the exothermic peak does not appeared. These transitions occur at a lower temperature as compared with fenugreek gum. The endothermic peak is due to the loss of water content in polymer (Bassi & Kaur, 2015). The disappearance of exothermic peak due to complete degradation of polymer backbone is observed. These peak shows low thermal stability as compared to fenugreek gum i.e. decreased availability of OH groups for intra-molecular hydrogen bonding. The Tg of AFG was also observed high in AFG (47.34°C) as compared to FG (47.95°C), indicating a high degree of Crystallinity of polymer.

DSC of Aminated tamarind gum:
The DSC thermograms of aminated tamarind, in that the aminated tamarind shows endothermic peak at 82.40°C and 394.50°C with heat of flow 1.002 w/g and 9.231 w/g or the exothermic peak does not appeared. The endothermic peaks are due to the loss of water content in polymer. The disappearance of exothermic peak was 365.35°C and heat of flow 9.639 w/g, due to complete degradation of polymer backbone. The melting point was showed by 276.49°C.

DSC of aminated xanthan gum:
The DSC thermograms of aminated xanthan gum, in that the aminated xanthan gum shows endothermic peak at 71.74°C and 541.66°C with heat of flow 1.644 w/g and 9.424 w/g or the exothermic peak does not appeared.

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Table 3: Flow properties of natural gum and derivetized gum

Polymer	Bulk density	Tapped density	Angle of repose	Compressibility index (%)	Hausner's ratio
Fenugreek Gum	0.37	0.41	33.6	18.6	1.22
Aminated Fenugreek Gum	0.39	0.45	35.9	13.33	1.15
Tamarind Gum	0.37	0.41	15.4	9.75	1.1
Aminated Tamarind Gum	0.41	0.55	18.2	25.45	1.34
Xanthan Gum	0.38	0.45	16.7	15.55	1.18
Aminated Xanthan Gum	0.43	0.52	19.1	17.3	1.2

*All value calculated in average of five reading.

The endothermic peaks are due to the loss of water content in polymer. The disappearance of exothermic peak was 430.05°C and heat of flow 11.82 w/g, due to complete degradation of polymer backbone. The melting point was showed by 277.20°C.

The X-ray ray differactograms (XRD):

The X-ray differactograms of aminated fenugreek gum, aminated tamarind gum and aminated xanthan gum. The diffractions curve of aminated fenugreek gum, aminated tamarind gum and aminated xanthan gum was typical of amorphous material with no sharp peaks.

Flow properties and physicochemical evaluation of aminated polymers:

The bulk density, tapped density and angle of repose of synthesized polymers were increased as compared to natural polymer due to reason of chemical modification was done in natural polymer. Compressibility index and Hausner's ratio describe the flow properties of natural polymers and derivetized polymers. Observations as per compressibility index the aminated fenugreek gum shows good flow properties as compare to natural fenugreek gum and aminated tamarind gum shows poor flow properties as compare to natural tamarind gum. In case of aminated xanthan gum compressibility index is partially an increases shows good flow property. The values of Hausner's ratio are < 1.25, shows good flow. Here all derivetized polymers show good flow properties except aminated tamarind gum. Evaluation parameters like bulk density, tapped density, angle of repose, compressibility and Hausner's ratio was carried out for the natural polymers and derivetized polymers and was found to be within the limit as given in Table 3.

Drug content and physical evaluation:

Drug content of the formulations was assayed spectrophotometrically at 215nm. Assayed content of drug in various formulations varied between 98.23% and 100.30% (average 99.35%). Tablet weights varied between 249.29mg and 250.30mg (average 249.93 mg), hardness between 3.5 and 5.5kg/cm^2 (average 4.46 kg/cm^2), thickness between 3.09 and 3.12mm and friability ranged from 0.49% and 0.87% (average 0.73%). Thus all the physical parameters of the compressed matrices were found to be practically within controls.

Fitting of data to model:

A three-factor, three-level Box-Behnken statistical experimental design as the RSM requires 15 experiments (Polynomial analysis). The independent variable and the response for all 15 experimental run are given in Table 2. Eleven batches showed the burst release *(Y1)* of less than 10% and the range of *Y1* for all batches was 6.69–12.41%. The ranges of other responses, Y2 (% dissolution after 60 min) and *Y3* (hardness of the tablets, kg/cm^2), were 21.84–37.53% and 3.5–5.5kg/cm^2, respectively. All the responses observed for 15 formulations and analyzed with polynomial equation of statistics analysis [17] were simultaneously fitted to first order, second order and quadratic models using Design Expert® and the comparative values of R^2, S.D. and % C.V. are given in Table 4 along with the regression equation generated for each response. Responses *Y1*, *Y2* and *Y3* were found to follow linear, quadratic and second order model respectively (Table 4, right column). Only statistically significant ($p < 0.05$) coefficients are included in the equations. A positive value represents an effect that favors the optimization, while a negative value indicates an inverse relationship between the factor and the response. It is evident that the Aminated fenugreek gum *(X1)*, Aminated tamarind gum *(X2)* and Aminated xanthan gum *(X3)* have negative effects on the responses *Y1* and *Y2* in the following order;

ATG (X2) > AFG (X1) > AXG (X3)

Coefficients with higher order terms or more than one factor term in the regression equation represent quadratic relationships or interaction terms, respectively. It also shows that the relationship between responses and factors is not always linear. Used at different levels in a formulation or when more than one factors are changed simultaneously, a factor can produce different degree of response. The interaction effect of *X1* was seen with *X2* and *X3* for response *Y2*; and between *X1* and *X3* for response *Y3*. *X2* also showed a higher quadratic effect as compared to *X1* on response *Y2*. Percentage burst release *(Y1)* and hardness of the tablets *(Y3)* were found to fit the linear and second order models, respectively. In absence of the quadratic effects, *Y1* was mainly dependent upon the amount of ATG. For *Y3*, the critical parameters were found to be the AFG and the ATG.

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Table 4: Summary of regression analysis of *Y1*, *Y2* and *Y3*

Model	R^2	Adjusted R^2	Predicted R^2	S. D.	% C. V.	Remark
Response (Y1)						
Linear model	0.8805	0.848	0.8081	0.7093	7.98	**Suggested**
Second order	0.9101	0.8427	0.7901	0.7214	8.12	-
Quadratic model	0.9653	0.9028	0.7965	0.5672	6.39	-
Response (Y2)						
Linear model	0.9441	0.9288	0.9046	1.29	4.1	-
Second order	0.9467	0.9067	0.8189	1.48	4.7	-
Quadratic model	0.9944	0.9842	0.96	0.6069	1.93	**Suggested**
Response (Y3)						
Linear model	0.7514	0.6835	0.5217	0.4569	10.23	-
Second order	0.9206	0.861	0.8048	0.3028	6.78	**Suggested**
Quadratic model	0.9255	0.7915	0.5532	0.3708	8.3	-
Regression equation of the fitted model [a]						
Y1=8.88-0.70X1-2.09X2-0.48X3						
Y2=32.91-2.29X1-5.68X2-0.97X3+0.20X1X3-0.005X2X3-0.92X1^2-1.89X2^2						
Y3=4.5+0.31X1+0.87X2+0.06X3+0.62X1X3						
[a] Only the terms with statistical significance are included.						

Table 5: Standardized main effects of the factors on the responses

Factor	Standardized main effect of the factors on the responses		
	Burst release (Y1) linear model	**Dissol. 60 min. (Y2) quadratic model**	**Hardness (Y3) second order model**
X1	2.801441	10.7008	2.919371
X2	8.339521	26.4928	8.174239
X3	1.919137	4.52615	0.583874
*X1*X2*	-	-	-
*X1*X3*	-	0.683756	4.128614
*X2*X3*	-	0.01648	-
*X1*X1*	-	2.92189	-
*X2*X2*	-	5.98494	-
*X3*X3*	-	-	-
R^2	88.05%	99.44%	92.06
p- value of lack of fit	0.6624	0.791	0.9678

[a] Only term with statistical significance are included

Standardized main effects and reliability of the models:

Standardized Main Effects (SME), presented in Table 5, SME were calculated by dividing the main effects with the standard error of the main effects [17],[25]. Only statistically significant ($p < 0.05$) values are given. The larger SME value of *X2* suggested the paramount importance of ATG on drug release. R^2-value signifies the percentage of variability in responses that are fitted to the models. In the present study, the high R^2-value of > 99% represents the reliability of the design. Additionally, the *p*-values of lack of fit were greater than 0.05, which further strengthened the reliability of the models (Table 5).

Contour plots and response surface analysis:

Two-dimensional contour plots and three-dimensional response surface plots are presented in Figure 1 which is very useful to study the interaction effects of the factors on the responses. These types of plots show the effects of two factors on the response at a time. In all the presented figures, the third factor was kept at a zero level. Figure 1 (B) and (C) exhibits a nearly linear relationship of factor *X3* with factors *X1* and *X2*, in the form of almost straight line. Response surface plots show the relationship between these factors even more clearly. However, factor *X1* and *X2* have non linear relationship Figure 1 (A) and Figure 1 (D) shows that 39.5 % drug is released after 60 min *(Y2)* when both the AFG and ATG are at lowest level and the decrease in % drug release was polymer concentration dependent. Also the ATG resulted in greater reduction in % release at 12 % level as compared to the AFG at 9% concentration. This indicates a slight non-linear trend between the factors *X1* and *X2*. Figure 1 (E) and (F) show an increasing trend for *Y2* upon the replacement of either of AFG or ATG with AXG.

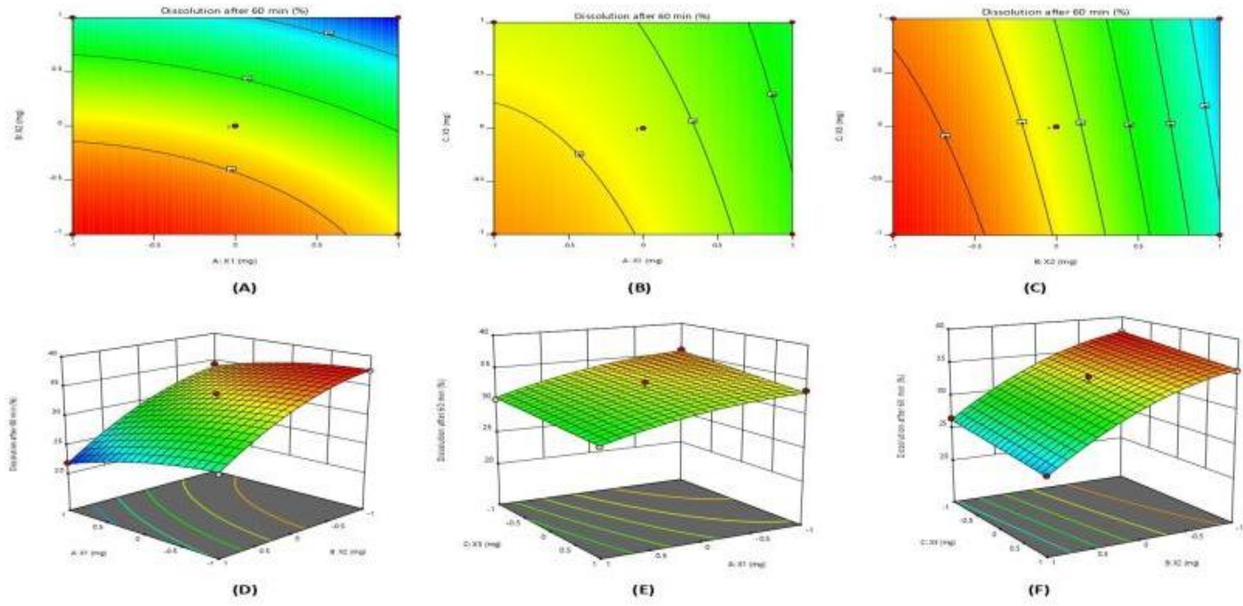

Figure1: Contour plot showing the effect of- (A) AFG (X1) and ATG (X2) on response Y2, (B) AFG (X1) and AXG (X3) on response Y2, (C) ATG (X2) and AXC (X3) on response Y2;
Response surface plot showing the effect of - (D) AFG (X1) and ATG (X2) on response Y2, (E) AFG (X1) and AXG (X3) on response Y2, (F) ATG (X2) and AXC (X3) on response Y2

Optimization:

The optimum formulation was selected based on the criteria of attaining the maximum hardness for tablets and applying constraints on Y1 ($10 \leq Y1 \leq 15$) and Y2 ($31 \leq Y2 \leq 35$). Upon 'trading off' various response variables and comprehensive evaluation of feasibility search and exhaustive grid search, the formulation composition with polymer levels of AFG- 37.40 mg, ATG- 10 mg and AXG- 24.91 mg, was found to fulfill the maximum requisite of an optimum formulation because of better regulation of % burst release and % dissolution after 1 hr time interval. The optimized formulation was found to release about 99.12% drug in sustained release manner for 12 hr. Study of the in vitro release profiles in 0.1 N HCl (for 2 hr) and in phosphate buffer, pH 6.8 (for 10 hr), of the formulations showed a burst release of 37.61% during 1 hr followed by a gradual release phase for about 10 hr. Figure 2 shows the complete dissolution profile of the optimized formulation. The optimization of the formulation was carried out from overlay plot. Overlay plot gives the area of interest or area of the experiment. In Figure 3 yellow region reflects the area of experiment.

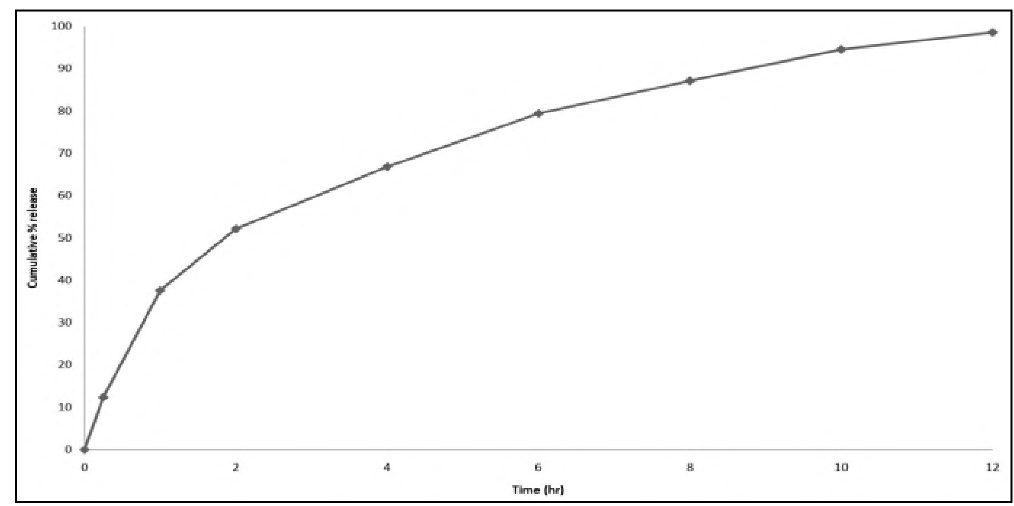

Figure 2: Dissolution profile of the optimized formulation.

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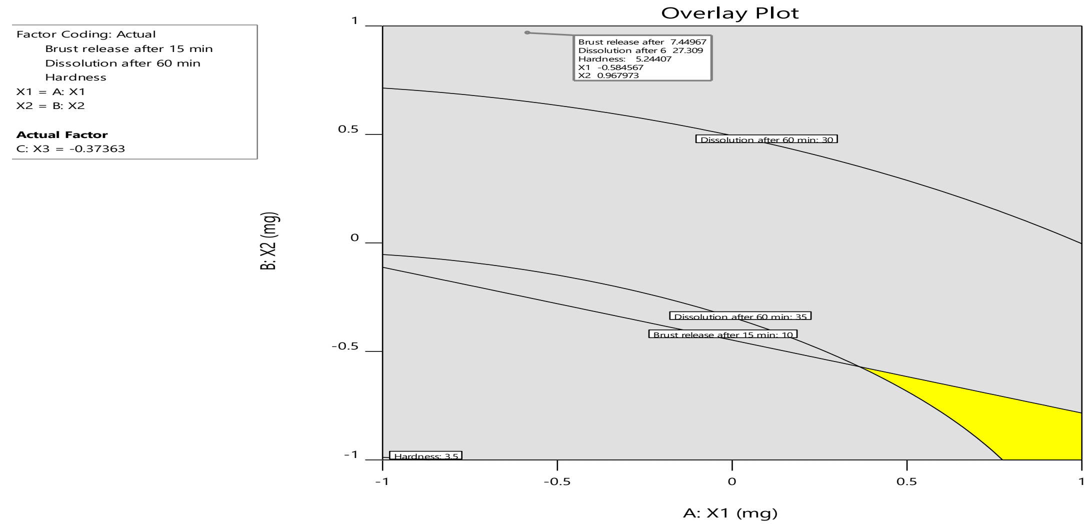

Figure 3: Overlay plot of optimized batch of formulation.

Validation of RSM result:

For all of the checkpoint formulations, the results of the physical evaluation and tablet assay were found to be within limits. Table 6 shows the composition of optimum checkpoint formulations, their predicted and experimental values of all the response variables, and the percentage error in prognosis. Linear correlation plots between the actual and the predicted response variables were plotted and the residual plots, showing the scatter of the residuals versus actual values, are presented in Figure 4. For validation of RSM results, the experimental values of the responses were compared with that of the anticipated values and the prediction error was found to vary between 0.7533% and 0.9541%. The linear correlation plots drawn between the predicted and experimental values demonstrated high values of R^2 (ranging between 0.9300 - 0.9950) indicating excellent goodness of fit ($p < 0.001$) [26]. Thus the low magnitudes of error as well as the significant values of R^2 in the present investigation prove the high prognostic ability of the RSM [20].

Table 6: Composition of optimum checkpoint formulation, the predicted and experimental values of response variables and percentage prediction error

Formulation composition (X1:X2:X3)	Response variable	Experimental value	Predicted value	Percentage prediction error
7.5:10.0:37.5	Y1 (%)	6.69	6.3108	0.3791
	Y2 (%)	24.39	24.3437	0.04625
	Y3 (kg/cm2)	5.5	5.4041	0.09583
22.5:10.0:25.0	Y1 (%)	8.13	7.6995	0.4304
	Y2 (%)	28.73	28.9125	-0.1825
	Y3 (kg/cm2)	5.5	5.4666	0.03333
7.5:10.0:37.5	Y1 (%)	7.23	7.2733	-0.0433
	Y2 (%)	26.48	26.2962	0.1837
	Y3 (kg/cm2)	5.5	5.2791	0.2208
22.5:30.0:25.0	Y1 (%)	9.63	10.272	-0.642
	Y2 (%)	34.27	33.9037	0.3662
	Y3 (kg/cm2)	4	3.9041	0.09583
7.5:20.0:12.5	Y1 (%)	12.41	11.4558	0.9541
	Y2 (%)	37.61	37.6562	-0.04625
	Y3 (kg/cm2)	3.5	3.5291	-0.0291
22.5:20.0:12.5	Y1 (%)	11.87	11.677	0.1929
	Y2 (%)	37.53	37.6662	-0.1362
	Y3 (kg/cm2)	3.5	3.2791	0.2208
7.5:20.0:37.5	**Y1 (%)**	**7.16**	**7.4945**	**-0.3345**
	Y2 (%)	**26.76**	**27.1262**	**-0.36625**
	Y3 (kg/cm2)	**5**	**5.0291**	**-0.0291**
22.5:20:37.5	Y1 (%)	9.27	9.1045	0.1654
	Y2 (%)	33.41	33.09	0.32
	Y3 (kg/cm2)	3.5	3.5916	-0.0916
15.0:10.0:12.5	Y1 (%)	8.13	8.8833	-0.7533
	Y2 (%)	32.21	32.9133	-0.7033
	Y3 (kg/cm2)	4	4.4666	-0.4666

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15.0:30.0:12.5	Y1 (%)	10.71	10.4933	0.2166
	Y2 (%)	35.54	35.7237	-0.1837
	Y3 (kg/cm2)	3.5	3.6541	-0.1541
15.5:10.0:37.5	Y1 (%)	8.56	8.662	-0.10208
	Y2 (%)	30.12	30.44	-0.32
	Y3 (kg/cm2)	4	4.0916	-0.0916
15.0:30.0:37.5	Y1 (%)	6.81	6.0895	0.7204
	Y2 (%)	21.84	21.70375	0.1362
	Y3 (kg/cm2)	5.5	5.6541	-0.1541
7.5:10:12.5	Y1 (%)	10.45	10.067	0.3829
	Y2 (%)	35.63	35.4475	0.1825
	Y3 (kg/cm2)	4.5	4.7166	-0.2166
7.5:10:12.5	Y1 (%)	7.33	8.8833	-1.5533
	Y2 (%)	32.79	32.9133	-0.1233
	Y3 (kg/cm2)	4.5	4.4666	0.0333
7.5:10:12.5	Y1 (%)	8.87	8.8833	-0.0133
	Y2 (%)	33.74	32.9133	0.8266
	Y3 (kg/cm2)	5	4.4666	0.5333

* In bold case value shows optimized batch

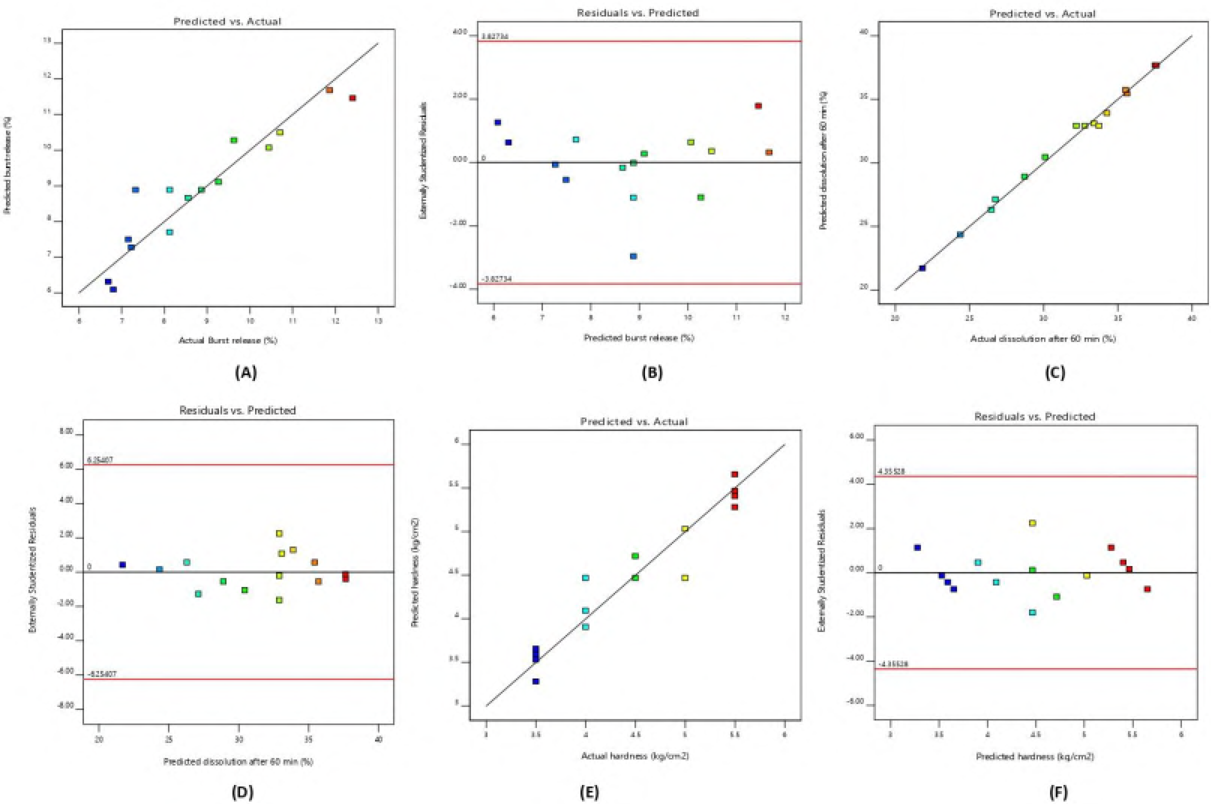

Figure 4: Linear correlation plots (A, C, E) between actual and predicted values and the corresponding residual plot (B, D, F) for various responses.

Swelling studies:

The swelling and erosion behavior of the optimized matrix tablet in 0.1N HCl and in PBS, pH 6.8, as a function of time, is shown in Figure 5. It can be observed that the hydrophilic matrix tablets underwent both swelling and erosion at the same time. The tablets achieved maximum swelling after 1 hr, which can be linked to the initial burst release of LP. Constant release can be obtained from such hydrophilic systems because of the simultaneous swelling and erosion of the matrix tablets. Constant release in such situations occurs because the increase in diffusional path length due to swelling is compensated by continuous erosion of the matrix. The cross-sectional SEM images of matrix tablets after 2 hr in acidic and 6 hr in basic media are shown in Figure 6 (A) (B). SEM study of the dissolving matrix tablets showed a uniform swelling of the matrix and further supported the fact of drug release by a diffusion process from the highly porous and swollen matrix tablets (figure 6).

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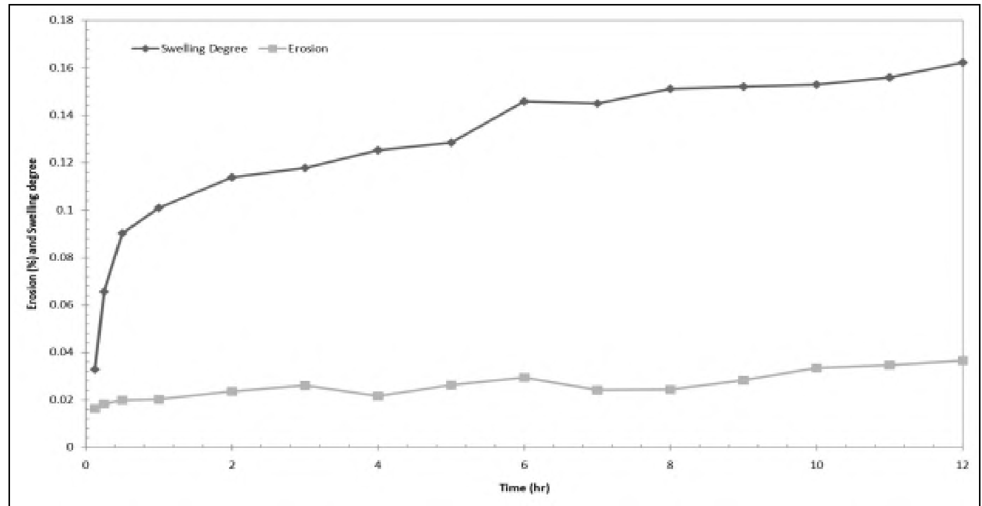

Figure 5: Erosion and swelling behavior of optimized formulation.

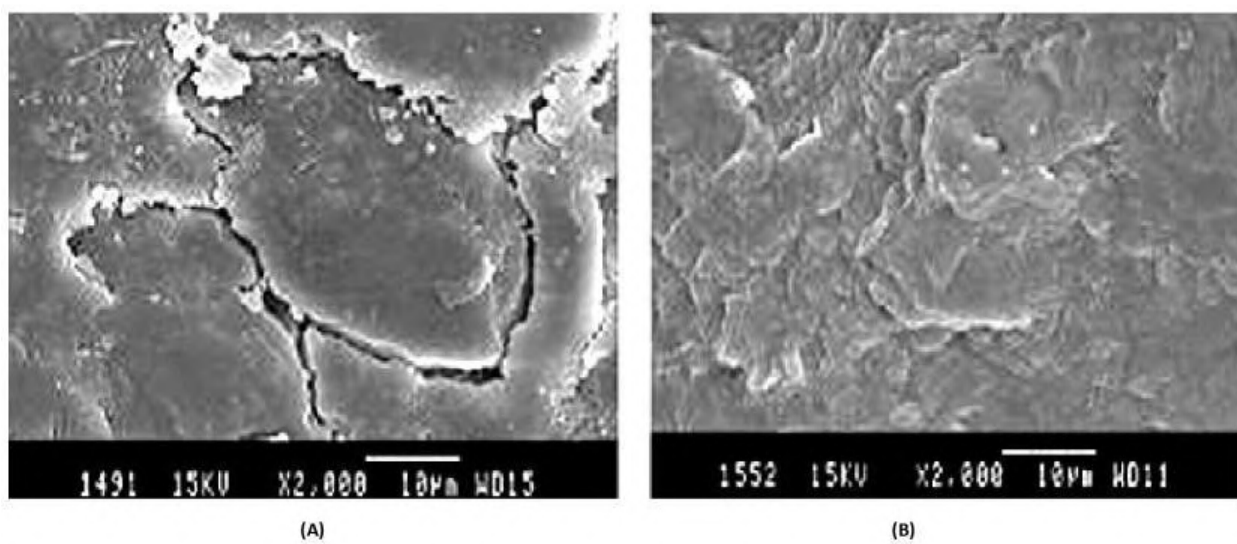

Figure 6: SEM photomicrographs showing surface topography of hydrated matrices in (A) acidic media, 2 hr (B) basic media, 6 hr.

Thermal properties:

DSC thermogram of the drug, excipients and the optimized formulation were recorded, in order to determine the thermal changes of polymers and drug before and after preparation. The characteristic endothermic peak of the drug at 255.46°C was observed in formulation also. However, the broadening of the drug peak in optimized formulation was related more to the impurities from excipients than physical interaction of the drug with the components.

Compatibility study of Losartan potassium by Fourier transform infrared (ATR-FTIR) spectroscopy:

FTIR spectra of the drug, excipients and the optimized formulation were recorded in range of 4000 – 400 cm^{-1}. LP showed some prominent and characteristic peaks at 3394 cm^{-1}, 1026 cm^{-1}, 1643 cm^{-1}, and 764 cm^{-1}, which could be assigned to stretching vibrations of O-H and C-O bond of primary alcohols, N=N stretching and C-Cl bond, respectively. In the optimized formulation, the presence of all the characteristic peaks of the LP indicates lack of any strong interaction between the drug and the excipients.

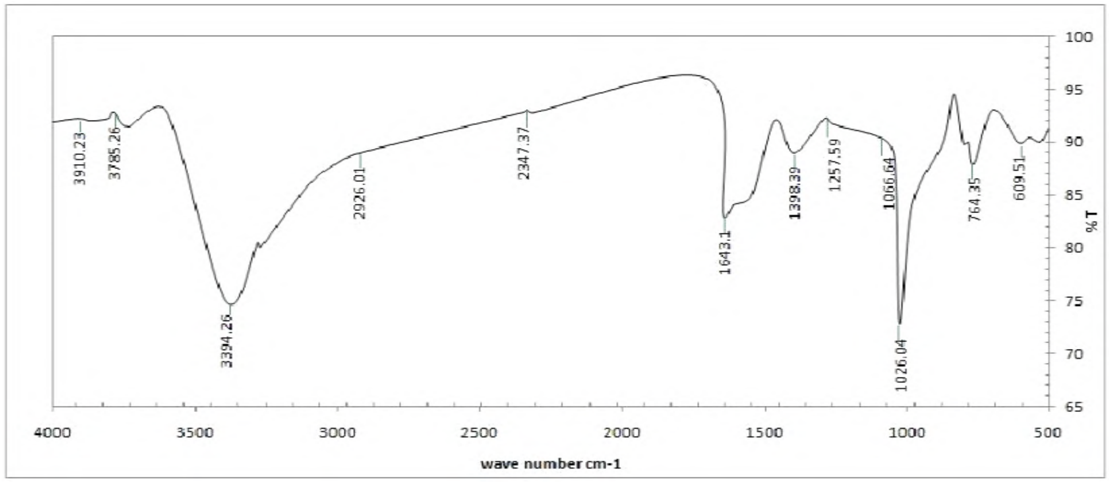

Figure 7: FTIR spectra of optimized formulation

CONCLUSION:

Release modifier polymer aminated fenugreek gum, aminated tamarind gum and aminated xanthan gum was synthesized and characterized. The synthesis polymers are characterized using ATR-FTIR, DSC and XRD studies. In this study, synthesis polymers are confirmed by ATR-FTIR study. The ATR- FTIR study of aminated fenugreek gum, aminated tamarind gum and aminated xanthan gum is confirmed by the appearance of a new peak at 3271 cm^{-1}, 1639.49 cm^{-1} and 2899.01 cm^{-1} respectively corresponding to NH2 group in the FTIR spectra of aminated fenugreek gum. Hydrophilic matrix tablets of LP with AFG, ATG and AXG were prepared and optimized using a three factor, three-level Box Behnken design. The quantitative effect of these factors at different levels on the release rate could be predicted by using polynomial equations. Linearity observed between the actual and predicted values of the response variables suggested the prognostic ability of the RSM design. The quadratic response surface methodology studied for the release rate helped in understanding the interaction effects between the combination and ratio of the three polymers. DSC and FTIR studies combined with the stability study of the optimized formulation proved the integrity of the developed hydrophilic matrix tablets. Thus, high degree of prediction obtained using RSM is quite efficient in optimizing drug delivery systems that exhibit non-linearity in responses.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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REVIEW ARTICLE

A Role of Herbal Drug as an Immunity Booster during Covid-19 Pandemic

Dhanashri Aware*, Sachin Rohane
Faculty of Pharmacy, Yashoda Technical Campus, Satara 415015, India.
*Corresponding Author E-mail: **awaredhanashri109@gmail.com**

ABSTRACT:
As the world scrambles to find a cure for COVID-19, Medical research network around the world is trying to find out treatment against the novel corona virus infection. In this pandemic condition there is a need for herbal remedies to boost the innate and acquired immunity to fight against corona virus. There are other certain ways to boost the "Immune System" such as active lifestyle, healthy diet, physical exercise, relaxation and sound sleep. Home remedies can be played a vital role as immunity modulator. Ayurveda treatises have described several herbal drugs which are used as different home remedies and are assume to be effective in COVID-19 therapeutics and immunity modulator as a preventive solution. That's why in this present study, an challenge is made to review such herbal drugs and identify its immune modulator effect against corona virus. Tulsi, Ginger, Clove, Dalchini, Turmeric, Garlic, Marich are most effective herbal drugs used as a home remedies to improve the immunity level naturally with speedy recovery in COVID-19 cases.

KEYWORDS: Covid-19, Immunity, Herbal drugs.

INTRODUCTION:

COVID-19, a global pandemic declared by WHO, is a highly infectious and severe acute respiratory disorder caused by a pathogenic virus called SARS-CoV-2 which is transmitted to humans via contact and feeding on infected animals. The COVID-19 clinical manifestations are very similar to viral pneumonia such as fever, fatigue, cough, shortness of breath, and other complications. According to reports obtained on WHO and NCDC websites as of 12th July 2020, the coronavirus breakout in Wuhan, a city in Hubei Province of China in November 2019 as spread to more than 200 countries in the world. This global pandemic has forced many nations to lock down their social activities which in turn have adverse effects on the economy[1].

Coronaviruses belong to the subfamily Coronavirinae in the family Coronaviridae of the order Nidovirales and can cause respiratory, digestive, and nervous system diseases in humans and many other animals[2].

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Which consists of four genera namely: Alpha, Beta, Gamma, and Delta coronavirus. It is currently thought that, SARS-CoV-2 has azoonotic origin and has secondarily acquired human-to-human spreading capacity. In particular, the acquisition of 1)Mutations in the receptor-binding area, 2) A polybasic furin cleavage site (RRRAR)at the junction of subdomain 1 and 2 of the spike protein and 3) A site of O-linked glycosylation in the same area, have enabled the virus to efficiently interact with high affinity(via its spike protein) with its bona fide cellular receptor (angiotensin-converting enzyme 2[ACE-2]), to become more virulent and pathogenic, while potentially evading immune responses through O-glycan epitope maskimg[3].

Morphology and genomic structure of HCoV:

Coronaviruses are spherical or pleomorphic, with diameter of 80-120nm. The virion surface is decorated with club like projections constituted by the trimeric spike (S). The viral envelop is supported by the membrane (M) proteins the most abundant structural protein. And a small transmembrane protein known as the envelop (E) protein is also present in a low amount in the envelope. The genomic RNA and phosphorylated

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nucleocapsid (N) protein form a spiral nucleocapsid, which is located within the envelope. The coronavirus genome is comprised of a single-stranded positive-strand RNA ranging from 27 Kb to 32 Kb in length. The genomic RNA is 5'-capped and 3'-polyadenylated and contains multiple open reading frames (ORFs). The invariant gene order is 5'- replicase-S-E-M-N-3', with numerous small ORFs (encoding accessory proteins) scattered among the structural genes[4].

Entry mechanism of human coronaviruses:

The life cycle of SARS-CoV-2 in host cells- begins when S protein binds to the cellular receptor ACE2. After receptor binding, the conformation change in the S protein facilitates viral envelope fusion with the cell membrane through the endosomal pathway. Then SARS-CoV-2 releases RNA into the host cell. Genome RNA is translated into viral replicase polyproteins pp1a and 1ab, which are then cleaved into small products by viral proteinases. The polymerase produces a series of subgenomic mRNAs by discontinuous transcription and finally translated into relevant viral proteins. Viral proteins and genome RNA are subsequently assembled into virions in the ER and Golgi and then transported via vesicles and released out of the cell[5].

Symptoms and effects of covid-19:

An infected COVID-19 patient can have two major states of infection, the asymptomatic state, and the symptomatic state. The symptomatic stage can develop into Acute Respiratory Disease Syndrome (ARDS) then rising infection can lead to multi- organ failure which can be fatal to the patient. An asymptomatic patient does not exhibit any symptoms of the disease due to high immunity but is still capable of infecting others, his state is extremely dangerous for the community and transmission of the virus. It is impossible to identify an asymptomatic patient without conducting an RT-PCR (Real-time polymerase chain reaction) test. Symptomatic patients exhibit varying level of severity of the disease, most patients display mild symptoms only like fever, cough, sore throat, headache, myalgia or severe symptoms like ARDS or organ failure. In the case of COVID-19, an extreme rise in inflammatory cytokines, monocytes, etc. leads to vasodilation. Which leads to the symptoms including shortness of breath, rapid breathing and bluish skin coloration[6].

Prevention:

The prevention and management are very important issues to control COVID-19. Therefore, there is a great need for the collective efforts of the public and the government. The regular and the proper care of the homes and hospitals are very important to control this calamity. The hand cleaning with soap and sanitizer, mouth and nose coverage with mask during sneezing and coughing are essential. Touching specific parts of the face like eyes, nose, and mouth without washing the hands should be avoided as these are entry points for the virus. Avoiding person-to- person contact. Regular cleaning of the surface by the disinfectants may control the virus outbreak. It is always better to avoid the interactions with anyone; suspecting respiratory problems symptoms like sneezing, coughing, breathing problem, etc. Screening has a vital role as a preventative measure to detect a potential health problem in an individual who doesn't have any signs and symptoms. Screening should be done in a multiphase level to aid further management of the disease[7].

Immunity:

The immune system refers to a collection of cells and proteins that function to protect the skin, respiratory passages, intestinal tract and other areas from foreign antigens, such as microbes (organisms such as bacteria, fungi, and parasites), viruses, cancer cells, and toxins. The immune system can be simplistically viewed as having two "lines of defense": innate immunity and adaptive immunity. Innate immunity represents the first line or defense to an intruding pathogen. It is an antigen-independent (non-specific) defense mechanism that is used by the host immediately or within hours of encountering an antigen. The innate immunity response has no immunologic memory and, therefore, it is unable to recognize or "memorize" the same pathogen should the body be exposed to it in the future. Adaptive immunity, on the other hand, is antigen- dependent and antigen-specific and, therefore, involves a lag time between exposure to the antigen and maximal response. The hallmark of adaptive immunity is the capacity for memory which enables the host to mount a more rapid and efficient immune response upon subsequent exposure to the antigen. Innate and adaptive immunity are not mutually exclusive mechanisms of host defense, but rather are complementary, with defects in either system resulting in host vulnerability[8].

Immunomodulators:

An immunomodulator may be defined as a substance, biological or synthetic, which can stimulate, suppress or modulate any of the components of the immune system including both innate and adaptive arms of the immune response. modulation of the immune system denotes to There are generally of two types immunomodulators based on their effects: immune suppressants and immune stimulators. Specific immunomodulators administered together with antigens to boost the immune response to the vaccine constituents. For instance, a planet origin saponin used in veterinary medicine. Whereas, non-specific immunostimulators offer a generalized state of resistance to pathogens or tumors. The concept of immunomodulation relates to nonspecific activation of the function and efficiency of macrophages, granulocytes, complement, natural killer cells and

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lymphocytes and also to the production of various effectors molecules generated by activated cells. It is expected that theses nonspecific effects give protection against different pathogens including bacteria, viruses, fungi etc. and constitute an alternative to conventional chemotherapy[9].

Our body temperature and wealth of nutrients provide an ideal home for these micro-organisms to thrive. The human immune system comprises innate and acquired immunity. Natural killer (NK) cells, complement system, macrophages, antigen presenting cells (APCs) and neutrophils make up the innate immune system and mounts an immediate non-specific response to foreign microbial agents. If microbes by-pass this primary defence, the acquired immune response, comprising humoral and cell mediated components, will then act to contain the invaders. The type of antigen (fungi, virus, bacteria, toxin) processed and presented by APCs to the CD4+ T cell determines the type of cytokines secreted, which in turn, determine the differentiation of helper T (TH) cells into TH1 or TH2 cells and B-cells to give immunoglobulin sub- types. TH1 response involves the activation of macrophages, which contain and destroy mycobacteria and fungal fungal pathogens. TH1 pathway also activates cell-mediated immunity. TH2 cells, on the other hand, effect immunoglobulin differentiation and antibody secretion, and therefore mediate humoral immunity. CD8 cytotoxic T cells induce apoptosis in antigen-laden cells[9].

Ayurveda purview:

Ayurveda is a comprehensive scientific medicinal system indigenous to India. The term Ayurveda means ‘knowledge of life’. Which comprises two Sanskrit words, Ayu (life) and Veda (knowledge or science). Four Vedas, considered as a the oldest Indian literature (5000-1000 BC) contain information about natural remedies. Ayurveda was established as fully grown medicinal system. Charaka Samhita (focussing on internal medicine) and Susruta Samhita (focussing on surgery) were written systematically and considered as classical text of Ayurveda. Vital details of Charaka Samhita and Susruta samhita were compiled together and updated additionally in Astanga Sangraha and Astanga Hrdaya. Some other ancient classics which include minor work of Ayurveda includes Madhava Nidana (focusing on diagnosis of disease), Bhava Prakasa (focussing on additional information related to plant and diet), arngadhara Samhita (focusing on formulation and dosage form). Ayurveda was divided into eight major clinical subdivisions-Kayacikitsa (internal medicine), Salya Tantra (surgery), salakya (diseases of supra- clavicular origin), Kaumarabhrtya (paediatrics, obstetrics and gynaecology), Bhutavidya (psychiatry), Agada Tantra (toxicology), Rasayana Tantra (rejuvenation and geriatrics), Vajikarana (aphrodisiology and eugenics)[10].

Concepts underpinning ayurvedic medicine:

The 3 basic principles, called doshas (vata, pitta, and kapha), are derived from 5 elements of Indian philosophy. Ayurveda’s doshas can be identified as regulatory control factors for fundamental physiologic processes in living systems that maintain their identity throughout biologic history: vata and its subdoshas regulating input/output processes and motion; pitta and its subdoshas regulating throughput, turnover, and hence energy; and kapha and its subdoshas regulating storage, structure, and lubrication. Factors such as food, activity, the climate and stress can, however, disrupt or destroy these functions. Ayurveda seeks to normalize body functions with varied techniques including advice onfood and activity, internal herbal preparations, purification treatments (panchakarma), and surgical methods (shalya chikitsa). Oral administration routes play a major role in influencing individuals’ doshas, via the ingestion of food, spices, and medicinal plants. These elements are influencing doshas in different ways: stabilizing, disturbing, and supporting the body’s healthy state[11].

Role of herbal drugs as immunity booster:

Plants are always the key source of drug or treatment strategy in different traditional medicinal systems. In recent years, many people are choosing to plant based medicines or products to improve their health conditions or as curative substance either alone or in combinations with others. According to the WHO, herbs or herbal products are used by the large number of populations for basic healthcare needs. Herbal medicine includes herbs, herbal materials (like plant parts) or preparations, processed and finished herbal products, active ingredients[10].

1. Tulsi:

Many in-vitro, animal and human experimental scientific studies showed that; due to presence of eugenol, phenolic compounds, linoleic acid, etc. compounds Tulsi has antimicrobial (including antibacterial, antiviral, antimalarial), anti-diarrheal, anti-oxidant, anti-inflammatory, hepatoprotective, cardioprotective, reno-protective, analgesic, antipyretic, immunemodulatory properties and is thus recommended as a treatment for a range of diseases including features like cough, fever, asthma, anxiety, diarrhea, gastric, cardiac and genitourinary disorders. Due to its anti-inflammatory and antioxidant properties, it protects against toxic chemical-induced injury, enhance the antioxidant enzymes and protect cellular organelles and membranes by clearing damaged free radicals. The compounds such as ursolic acid, carnosol, rosmarinic acid, cirsilineol, apigenin, eugenol, and cirsimaritin present in O. sanctum increase haemoglobin concentration, enhance SRBC agglutinin titers, decrease cyclo-oxygenase (CoX)-2 and lipoxygenase (LOX)-5 enzymes activity, suppress NF-

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kB classical pathway, up regulation of IL-2, IFN-g and TNF-a, down regulation of IL-1b and produce of SRBC antigen-specific antibodies, which represent a major defense mechanism to assess T-cell-dependent antibody responses i.e. Tulsi by enhancing immune response boost the defense mechanism against the infection. Several studies have shown that Tulsi (aqueous and methanol extract of leaf and seed oil) besides improving vital capacity also is an immune-modulator and regulator as it enhances immune response by increasing T-helper and NK cells; phagocytic activity and index with the rise in lymphocyte count, neutrophil count and antibody titer [12].

2. Ginger:

Ginger is the rhizome of Zingiber officinale Roscoe in the family Zingiberaceae and has been used as a food, spice, supplement and flavoring agent and in traditional medicines for more than 3000 years in countries. Ginger has been used in traditional medicines to treat diseases and symptoms, such as colds, headache, nausea, upset stomach, diarrhea, arthritis and rheumatism, or used as a carminative, antiflatulent and digestant. Furthermore, ginger is known to have pharmacological activity against natural, chemical and radiation-induced toxicities, such as radioprotective, hepatoprotective, nephroprotective, neuroprotective, gastroprotective and reproductive-system-protective effect. The bioactive compounds of ginger such as nevirapine, b-sitosterol, 6 gingediol, germacrene, methyl-6-shogaol, 6-gingerol, a-linalool, 6-shogaol, gingerdion, zingiberene, etc., are known to inhibit viral replication; among these the most potent inhibitors of reverse transcriptase (RT) enzyme is b-sitosterol, which is predicted to be used as non nucleoside reverse transcriptase (NNRTIs) HIV-1 inhibitors. It is reported that Ginger contains TNF-a which is also known as an anti-influenza cytokine.The rhizome of Ginger and its main components like gingerols, shogaols, etc inhibit prostaglandin and leukotriene biosynthesis, inhibit cyclooxygenase and lipoxygenase activities, inhibits the synthesis of pro-inflammatory cytokines such as IL-1, TNF-a, and IL-8 without any significant effect in IL-6 levels; inhibit the excessive production of NO, PGE (2), TNF-a, and IL-1beta, reduce the elevated expression of NFkB and TNF-a, downregulate inflammatory iNOS and COX-2 gene expression, inhibit thromboxane synthetase, raise levels of prostacyclin without a concomitant rise in PGE 2 or PGE 2 alpha, inhibit platelet aggregation, decrease age-related oxidative stress markers and enhance

Fibrinolysis. The concentration of IgM and eosinophil count in non-smokers was significantly increased in a comparative study of the effect of ginger extract among male smokers and non-smokers, whereas the concentration of hemoglobin and lymphocyte count in smokers was strongly increased. This indicates that in non-smokers, ginger results in a stronger antibody response or humoral immunity than in smokers[12].

3. Clove:

Cloves are an aromatic herb that has many useful purposes. Approximately, 72-90% of the essential oil extracted from cloves has Eugenol. Other are Acetyl eugenol, Beta-caryophyllene and vanillin, Crategolic acid, tannins, gallotannic acid, methyl salicylate (painkiller), Flavonoids eugenin, kaempferol, Triterpenoids like oleanolic acid. The dried buds of cloves contain antiseptic, and anti-fungal agent. It also holds aphrodisiac and circulation-stimulating capacities. The oil of cloves has been used in a variety of health conditions including indigestion, generalized stress, parasitic infestations, cough, toothaches, headache, and blood impurities. Clove is used extensively in dental care for relieving toothache, sore gums and oral ulcers. Gargling with clove oil can also aid in sore throat conditions and bad breathe. Clove oil clears the respiratory passages, acting as an expectorant for treating many upper-respiratory conditions including colds, bronchitis, sinus conditions, cough and asthma. Not only purifies the blood, but also aids in stabilizing blood sugar levels, and may have benefits for diabetic individuals. Clove's antiviral and cleansing properties purify the body, augmenting our resistance to disease[13-16].

4. Dalchini:

The Cinnamon popularly known as Dalchini (Cinnamomum zeylanicum), belongs to the family Lauracae. Cinnamon has also been used for its medicinal properties for thousands of years. Made from the inner bark of the cinnamomum tree, its use has been dated as far back as ancient Egypt. Cinnamon is an immune simulator, protecting the body from bacterial or viral attacks. It helps your body fight infections and repair tissue damage. All the antioxidants are super powerful when it comes to bringing those anti-inflammatory properties. Cinnamon also gives us manganese, calcium, fiber and iron. Cinnamon also fights inflammation and helps ward off infections and herbal damaged tissue. Containing large amounts of polyphenol, cinnamon outranked "superfoods" like garlic and oregano in a study comparing the antioxidant, antitumor, antihypertensive, antilipemic, antidiabetic, gastroprotective, and immunomodulatory effects[17]. In addition to being lipid lowering and cardiovascular-disease- lowering compound, cinnamon has also reported to have activities against neurological disorders, such as Parkinson's and Alzheimer's diseases. Cinnamon is a coagulant and prevents bleeding. Cinnamon also increases the blood circulation in the uterus and advance tissue regeneration[18].

In one study, cinnamon at high dose (100mg/kg) showed immuno-stimulant activity as it significantly increased

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the phagocytic index, serum immunoglobulin levels and antibody titer and decreased the percentage reductions in neutrophil count. Cinnamon low dose (10mg/kg) increased serum immunoglobulin levels only. This showed that high dose increases both cell mediated and humoral immunity whereas low dose showed effect only on humoral immunity. The studies also suggest that cinnamaldehyde can act as a strong regulator of monocyte/macrophagemediated immune responses by inhibition of PI3K, PDK1 and NF-kB activation of signaling components. In addition to this, by the activation of CD29 and CD43, it blocked cell migration cellecell adhesion induced but not cell-fibronectin adhesion and it was able to suppress both the production of nitric oxide (NO) and up regulation of surface levels of co-stimulatory molecules (CD69 and CD80) and pattern recognition receptors (TLR2 and CR3).

5. Turmeric:

Turmeric (Curcuma longa), also known as “Indian saffron” due to its brilliant yellow colour, is a spice herb, member of the ginger family (Zingiberaceae) native to the Indian subcontinent and Southeast Asia, having more than a two centuries old scientific history. Turmeric obtained from ground-dried root contains different percentages of volatile and non-volatile oils, proteins, fats, minerals, carbohydrates, curcuminoids and moisture. Commercially available curcumin is a combination of three molecules, together called curcuminoids. Curcumin is the most represented (60–70%), followed by demethoxycurcumin (20–27%) and bisdemethoxycurcumin (10–15%). Besides curcuminoids, the other active components of turmeric include sesquiterpenes, diterpenes, triterpenoids[19].

Turmeric has various useful properties with antioxidant activities. Turmeric has anti-inflammatory, anticancer, anti-diabetic, hypolipidemic, antimicrobial, anti- fertility, anti-venom, hepatoprotective, nephroprotective, anticoagulant property. The plant has also shown to possess anti HIV activity to combat AIDS[17]. The immunomodulatory abilities of curcumin arise from its interaction with various immunomodulators, including not only cellular components, such as dendritic cells, macrophages and both B and T lymphocytes, but also molecular components involved in the inflammatory processes, such as cytokines and various transcription factors with their downstream signalling pathways. Curcumin supplementation in rabbit diet (2,4and 6g/kg) significantly increased serum levels of IgG and IgM, thus suggesting that curcumin can also improve immune response[20].

6. Garlic:

Garlic (Allium sativum) is bulbous perennial plant with a powerful onion such as aroma and pungent taste that has been used as flavoring agent, condiment, and for medicinal purposes for over 5,000 years. Garlic contains a variety of bioactive constituents includindg sulfur compounds such as alliin, allicin, ajoene, allylpropyl disulfide, diallyl disulfide (DADS), diallyltrisulfide (DATS), S-allylcysteine (SAS); peroxidases and alliniase like enzyme, amino acids and important trace elements like Se, Ge and Te. Garlic is frequently used to treat aches and pains, leprosy, diarrhea, infections, dandruff, respiratory disorders. Garlic has been employed for management of blood pressure, atherosclerosis, high cholesterol, heart attack and coronary heart disease. Aged garlic has more potent immunomodulatory effects than raw garlic. Garlic is an effective therapeutic candidate to prevent the recurrent aphthous ulcer. Conditions like gout, rheumatoid arthritis, osteoarthritis, diabetes, allergic rhinitis, traveler’s diarrhea, bacterial and fungal infections, cold and flu are also known to cured by garlic. Other uses of garlic include treatment of fever, whooping cough, headache, stomach ache, sinus congestion, psoriasis, hair loss and hemorrhoids[21].

7. Marich:

It has been also found to increase bioavailability, thus enhance the therapeutic efficacy of many drugs, vaccines and nutrients and have immune-modulatory, anti-oxidant, antiplatelets, antihypertensive, anti-asthmatic, antipyretic, analgesic, anti-carcinogenic, anti-inflammatory, anti-diarrheal, antispasmodic, anxiolytic, antidepressants, hepatoprotective, anti-ulcer, anti thyroids, antiapoptotic, anti-metastatic, antimutagenic, antibacterial, antifungal and anti-amoebic properties. The extract and its constituents like piperine, regulate the balance of the cytokines production of Th1, Th2, Th17, and Treg cells, reduce the accumulation of inflammatory cells, inhibit the expressions of GATA3, IL-4, IL-6, IL-1b, RORgt, IL-17A and TNF-a, increase INF-g and IL-10 secretions in BALF (Broncho-alveolar lavage fluid) and increase macrophage activation and T and B cell proliferation. Beside this, Marich possess cytotoxic activity, suppresses the levels of total IgE, anti-OVA IgE, anti-OVA IgG1 and histamine release in serum, ameliorates fibrosis and infiltration of inflammatory cells, inhibits the allergic responses, inhibitsTh2/Th17 responses and mast cells activation, inhibits NF-kB, c-Fos, cAMP response element-binding (CREB) and activated transcription factor (ATF-2); suppresses PMA-induced MMP-9 expression, inhibits PKCa/extracellular signal regulated kinase (ERK) 1/2 and reduces NF-kB/AP-1 activation. In addition, piperine also inhibits the Pglycoprotein (P-gp) and CYP3A4 functions. Piper nigrum is found to have dose dependent antifertility effects on mice[22].

CONCLUSION:

COVID-19 viral spectre outbreak is spreading across different countries at an increasingly alarming rate.

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Currently, yet no any vaccine or medicine could be developed to cure COVID-19 and Scientist also utilizing hydroxychloroquine to treat COVID-19 but could not get positive response and has side effect. Immune systems in body play an important role to fight against unhealthy environment and microbes such as virus, bacteria, fungus etc. In the current pandemic infection of COVID-19 it is clear that those with weak immune system are highly susceptible to this infection and worst outcomes. In the case of infectious pandemics like this "prevention is always better than cure". In this regard immune enhancing herbs may definitely be helpful for the body to fight COVID-19 infection. Tulsi, Ginger, Clove, Dalchini, Turmeric, Garlic, Marich these botanical plants having low cost, minimum toxicity and almost found everywhere in country, it has potential to enhance immunity to fight against COVID-19 and other infectious disease and play an important role to becomes fit and healthy India and world.

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REVIEW ARTICLE

Acute Toxicity study of Synthesized drug and Herbal Product

Tanuja Yadav*, Sachin Rohane
Faculty of Pharmacy, Yashoda Technical Campus, Satara 415015, India.
*Corresponding Author E-mail: **tanujayadav711@gmail.com**

ABSTRACT:
Acute toxicity study describes the adverse effects of a substance that result either from a single exposure or from multiple exposures in a short period of time. Whenever an investigator administered a chemical substance or herbal drug to a biological system different types of interactions can occur and a series of dose-related responses result. In most cases these responses are desired and useful, but there are a number of other effects which are not advantageous. These may or may not be harmful to the patient. Acute toxicity study is involved in estimation of LD_{50}. Also it determines the therapeutic index i.e ratio between the lethal dose and the pharmacologically effective dose in the same strain and species. This article Review the methods so for utilized for the determination of acute toxicity.

KEYWORDS: Acute toxicity study, Therapeutic index, Biological system.

INTRODUCTION:
The amount of pharmacological substances, chemical and herbal drugs being used in the human community today have increased to almost an innumerable amount. These may be presented today in the form or as constituents of food substances medicines, beverages, otherindustrial and household products. However these chemicals or herbal drug may result in chronic toxicity in the living system when used over a long period of time or acute toxicity may also occur when large quantities capable of eliciting immediate toxic effect are used. These effects may be mild or severe depending upon nature of the substance.

Acute toxicity is defined as the unwanted effect that occurs either immediate or at a short time interval after a single or multiple administration of such substance within 24 hours. The unwanted effect is any effect is any effect that produces functional impairments in organs and/or biochemical lesion which could alter the functioning of the organism in general or individual organs [1-2].

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Toxicity is the fundamental science of poisons. The organization for Economic and Development (OECD) mentioned acute toxicity as the advance effects occurring within short time of oral administration. Phychochemical interactions of poisons leads to the injury or death of living tissues. Toxicology is like science and an art like medicine. It includes observational data gathering and data utilization to predict outcome of exposure in human and animals. Acute toxicity is distinguished from chronic toxicity, which describes the adverse health effects from repeated exposures, often at lower levels to a substance over a longer time period [3-4].

Acute toxicity (LD_{50}) test:
Acute systemic toxicity evaluates the adverse effects that occur following exposure of organisms to a single or multiple doses of a test substance within 24 hours by a known route (oral, dermal or inhalation). After administration, the test substance is absorbed and distributed to various parts of the body before it elicits systemic adverse effect. The regulatory body requires the acute toxicity test report for labelling and classification of substances for human use.

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The LD_{50} (median lethal dose) test was introduced in 1927 by J.W. Trevan to estimate the dose of a test substance that produces 50% death in a given species of animals. It is usually the first test conducted for every chemical before further toxicity tests are carried out. It is used for estimating the potential hazards of chemicals on humans. Although its major endpoint is death, non-lethal acute effect may occur as sign of toxicity depending on the chemical being tested.

Assessment of the acute toxic potential of substances is required to determine their adverse effects that might occur due to accidental or deliberate short-term exposure. Result from acute toxicity test serve as a guide in dosage selection for long term toxicity studies are well as other studies that involve the use of animals.

From the result of an acute toxicity test, a conclusioncan be made on the toxicity status of the substance. As depicted in table 1, substance with LD_{50} below 5 mg/kg are classified to be highly toxic while substance with LD_{50} above 15,000 mg/kg are termed relatively harmless [5-8].

Table 1: Classification of LD_{50} based on dose range.

LD_{50}	Classification
<5 mg/kg	Extremely toxic
5-50 mg/kg	Highly toxic
50-500 mg/kg	Moderately toxic
500-5000 mg/kg	Slightly toxic
5000-50000 mg/kg	Practically non-toxic
>15000 mg/kg	Relatively harmless

OECD Guideline for Acute Oral Toxicity:

OECD Guideline for the testing of chemicals are periodically reviewed in the light of scientific progress or changing assessment practices.The conventional acute oral toxicity test (formerly OECD Test Guideline 401)is the most heavily criticized test in terms of animal welfare and this concern was the driving force behind the development of three alternative tests for acute oral toxicity (Test Guideline 420,423,425).Anticipating the presence of validated alternatives, Member countries took the initiative to plan the deletion of Guideline 401[9-12].

A Nominated Expert Meeting (Rome 1998) and an Expert Consultation Meeting, (Arlington 1999) were convened to determine the acute oral toxicity data requirement needs of Member countries and to assess the capabilities of the alternatives to meet these needs. On the basis of these technical discussions, the 29th Joint Meeting concluded in June 1999 that not all data needs could be met by the alternatives (and not always by Guideline 401). The Joint Meeting decided that Guidelines 420,423 and 425 should be revised to meet regulatory needs of member of countries.

The revision of Guidelines 420, 423, and 425 was completed in 2000 following a second Expert Consultation Meeting (Paris 2000) and process of deletion of guideline 401 was started[13-15].

OECD Guideline 420:
Acute Oral Toxicity-Fixed Dose Procedure:

The method provides information on the hazardous properties and allows the substance to be ranked and classified to the Globally Harmonised System (GHS) for the classification of chemicals which cause acute toxicity.

Principle of the test:

Group of animals of a single are dosed in a stepwise procedure using thefixed doses of 5, 50, 300 and 2000 mg/kg (exceptionally an additional fixed dose of 5000 mg/kg may be considered). The initial dose level is selected on the basis of a sighting study as the dose expected to produce some signs of toxicity without causing severe toxic effects or mortality. Further groups of animals may be dosed at higher or lower fixed doses depending on the presence or absence of signs of toxicity or mortality. This procedure continues until the dose causing evident toxicity or no more than one death is identified or when no effect are seen at the highest dose or when death occur at the lowest dose.

Description of the method:

1. **Selection of animal species:**

The preferredrodent species is the rat. Normally females are used. When the test is conducted in males, adequate justification should be provided. Females should be nulliparous an non pregnant. Each animal, at the commencement of its dosing, should be between 8 and 12 weeks old and its weight should fall in an interval within ± 20 % of the mean weight of any previously dosed animals.

2. **Housing and feeding condition:**

The temperature of the experimental animal room should be 22 c^0. Although the relative humidity should be at least 30% and preferably not exceed 70%.Lighting should be artificial ,the sequence being 12 hours light ,12 hours dark . For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water.

3. **Preparation of animals:**

The animals are randomly selected, marked to permit individual identification and kept in their cages for at least 5 days prior to the start of dosing to allow for acclimatisation to the laboratory conditions.

4. **Preparation of doses:**

Test substances should be administer in a constant volume over the range of doses. In case of liquid end product or mixture is to be tested –use undiluted test

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substance i.e at a constant concentration .In either case,the maximum dose volume for administration must not exceeded .In rodents , the volume should not normally exceed 1ml /100g of body weight however in case of aqueous solution 2ml/100g body weight can be considered. For vehicles other than water the toxicological characteristics of the vehicle should be known.

Procedure:

1. Administration of doses:

By gavage using a stomach tube or suitable incubation canula(unusual circumstance –fraction of doses).Fasted prior to dosing. Weigh animals and administer the test substance. Withheld food for a further 3-4 hours in rats or 1-2 hours in mice (in case of fraction of doses).

2. Sighting study:

The purpose of the sighting study is to allow selection of the appropriate starting dose for the main study. The substance is administered to single animals in a sequential manner following the flow charts.The starting dose for the sighting study is selected from the fixed dose levels of 5,50,300 and 2000 mg/kg as a dose expected to produce evident toxicity. Also, evidence from in vivo and in vitro data from the same chemical and from structurally related chemicals. In the absence of such information, the starting dose will be 300 mg/ kg. At least 24 hours will be allowed between the dosing of each animal. Consideration of use of an additional upper fixed dose level of 5000 mg/kg. In cases where an animal tested at the lowest fixed dose level (5 mg/kg) in the sighting study dies, the normal procedure is to terminate the study and assign the substance to GHS category. For further confirmation use supplementary procedure.

3. Main study:

Numbers of animals and dose levels

Select dose from sighting study. Perform study on a total of five animals of one sex at each level including animals tested in sighting study. Time interval between dosing at each level depends on onset, duration and severity of toxic signs. Treatment of animals at the next dose should be delayed until one is confident of survival of the previously dosed animals. To check delayed Toxicity-A period of 3 or 4 days between dosing at each dose level is recommended. In case of inconclusive response time interval may be adjusted as appropriate. Fixed dose of 5000 mg/kg procedure outline in Annex 4.

4. Limit test:

Performed when information indicating that the test material is likely to be nontoxic i.e. having toxicity only above regulatory limit doses. How information about the toxicity of the test material can be gained?. Using the normal procedure test will be done [16].

OECD Guideline 423:

Acute Oral Toxicity-Acute Toxic Class Method:

The acute toxic class method is based on biometric evaluation with fixed doses, adequately separated to enable a substance to be ranked for classification purposes and hazard assessment. The method as adopted in 1996 was extensively validated in vivo against LD_{50} data obtain from the literature, both nationally and internationally.

Principle of the test:

It is the principle of the test that, based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step i.e.

- No further testing is needed,
- Dosing of three additional animals with the same dose.
- Dosing of three additional animals at the next higher or the next lower dose level.

Description of the method:

1. Selection of animal species:

The preferred rodent species is the rat. When the test is conducted in males adequate justification should provided. Healthy young adult animals of commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant. Each animal, at the commencement of its dosing, should be between 8 and 12 weeks old and its weight should fall in an interval with in ±20%of the mean weight of any previously dosed animals.

2. Housing and feeding conditions:

The temperature in the experimental animal room should be 22^0C. Although the relative humidity should be at least 30% and preferably not exceed 70%. Lighting should be artificial, the sequence being 12 hours light, 12hours dark for feeding conventional laboratory diets may be used with an unlimited supply of drinking water.

3. Preparation of animals:

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

4. Preparation of doses:

Test substances should be administer in a constant volume over the range of doses. In case of liquid end product or mixture is to be tested-use undiluted test substance i.e. at a constant concentration. In either case,

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the maximum dose volume for administration must not exceeded. In rodent, the volume should not normally exceed 1 ml/100 g of body weight however in case of aqueous solution 2 ml/100 g body weight can be considered. For vehicles other than water the toxicological characteristics of the vehicle should be known.

Procedure:

1. Administration of doses:

The test substance is administered in a single dose by gavage using a stomach tube or suitable intubation canula. Single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 hours. Animal should be fasted prior to dosing. Following the period of fasting, the animals should be weighed and the test substance administered. After the substance has been administered, food may be withheld for a further 3-4 hours in rats or 1-2 hours in mice.

2. Number of animals and dose levels:

Three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels 5, 50, 300 and 2000 mg/kg body weight. The starting dose level should be that which is most likely to produce mortality in some of the dosed animals. The flow charts of Annex 2 describe the procedure that should be followed for each of the starting doses.

When available information suggests that mortality is unlikely at the highest starting dose level, then a limit test should be conducted. When there is no information on a substance to be tested, for animal welfare reasons it is recommended to use the starting dose of 300 mg/kg body weight.

The time interval between treatment groups is determined by the onset, duration, and severity of toxic sighs. Treatment of animals at the next dose, should be delayed until one is confident of survival of the previously dosed animals. Exceptionally, and only when justified by specific regulatory needs, the use of additional upper dose level of 5000 mg/kg body weight may be considered.

3. Limit test:

The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic i.e. having toxicity only above regulatory limit doses. Information about the toxicity of the test material can be gained from knowledge about similar tested compounds or similar tested mixtures or products, taking into consideration the identity and percentage of components known to be of toxicological significance. In those situation where there is little or no information about its toxicity, or in which the test material is expected to be toxic, the main test should be performed.

A limit test at one dose level of 2000 mg/kg body weight may be carried out with six animals. Exceptioally a limit test at one dose level of 5000 mg/kg may be carried out with three animals. If test substance-related mortality is produced, further testing at the next lower level may need to be carried out [17].

OECD Guideline 425:

Acute Oral Toxicity: Up and Down procedure-

This test procedure is of principal value in minimising the number of animals required to estimate the acute oral toxicity of a chemical and in estimating a medium lethal dose. The medium lethal dose allows for comparison with historical data. In addition to the observation of mortality, LD_{50} allows the observation of signs of toxicity. The latter is useful for classification purpose and in the planning of additional toxicity tests.

Principle of the test:

Animals are dosed, one at a time, at 24 hours intervals. The first animal receives a dose at the level of the best estimate of the LD_{50}. Depending on the outcome for the previous animal, the dose for the text animal is adjusted up or down. If an animal survives, the dose for the next animal is increased; if it dies, the dose for the next animal is decreased. After reaching the reversal of the initial outcome i.e. the point where an increasing dose pattern is reversed by giving a smaller dose, four additional animals are dosed following the same UDP. The LD_{50} is calculated using the method of maximum likelihood.

Description of the method:

1. Selection of animal species:

The preferred rodent species is the rat. In the normal procedure female rats are used. When there is adequate information to infer that males are more sensitive, they should replace females in the test. The females should be nulliparous and non-pregnant. At the commencement of the study, the weight variation of the animals should be minimal and not exceed ±20% of the mean weight for each sex. The test animals should be characterised as to species, strain source, sex, weight and age.

2. Housing and feeding conditions:

The temperature in the experimental animal room should be 22^0 C. Although the relative humidity should be at least 30% and preferably not exceed 70%. Lighting should be artificial, the sequence being 12 hours light and 12 hours dark. For feeding conventional laboratory diets may be used with an unlimited supply of drinking water.

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3. Preparation of animals:

The animals are uniquely identified and kept in their cages for at least five days prior to dosing for acclimatisation to the laboratory conditions. Animals demonstrating signs of spontaneous disease or abnormality prior the start of the study are eliminated from the study.

4. Preparation of doses:

When necessary, the test substance is dissolved or suspended in a suitable vehicle. It is recommended that, whenever possible, the use of an aqueous solution or suspension be considered first, followed by consideration of a solution or emulsion in oil and then by possible solution in other vehicle. For vehicles other than water, toxicity of the vehicle must be known.

Procedure:

1. Full test:

Individual animals are dosed in sequence at 24 h intervals, one at time and then observed for a minimum of 24 h. However, the time intervals between dosing should not be fixed rigidly and may be adjusted as appropriate, in case of delayed mortality. The first animal is dosed at the toxicologist's best estimate of the LD_{50}. If the animal survives, the second animal receives a higher dose, unless the limit dose was used as the starting dose. If the first animal dies or appears moribund the second animal receives a lower dose. Moribund state is characterised by symptoms such as shallow, laboured or irregular respiration, muscular weakness or tremors, absence of voluntary response to external stimuli, cyanosis and coma, Criteria for making the decision to humanely kill moribund and severely suffering animals are the subject of a separate Guidance Document. Animals killed for humane reasons are considered in the same way as animals that died on test.

For selecting the starting dose, all available information should be used, including information on structure-activity relationships. When the information suggests that mortality is unlikely then a limit test should be conducted. When there is no information on the substance to be tested, for animal welfare reasons it is recommended to use the starting dose of 200 or 500 mg/kg body weight. The dose for each successive animal is adjusted up or down, depending on the outcome of the previous animal. If feasible, a dose progression factor of 1.3 is used. Other factors may be used, if justified. After reaching the reversal of the initial direction, four additional animals are dosed using the same UDP. This is the end of the normal test.

2. Limit test:

Doses should not exceed 2000 mg/kg which is considered the upper limit dose. When the first animal is dosed with the upper limit dose and survives, the second animal receives the same dose. When total of three animals have been dosed with the limit dose and no deaths have occurred, then three animals of the other sex should be tested at the limit dose level. If there is again no lethality, the test can be terminated.

3. Optional testing:

Information from one sex may be adequate toxicity. However, if found desirable, comparability of response in the other sex can be evaluated by administering to generally not more than 3 animals, dose above and below the estimated LD_{50}. The point intermediate between doses where responses change can be taken as an appropriate estimate of the lethal dose.

4. Administration of doses:

The test substance is administered in a single dose by gavage, using an oral dosing needle or rubberised tubing. The animal should be fasted prior to dosing by withholding food overnight. Fasted body weight of each rat is determined and the dose is calculated according to the body weight. After dosing food may be withheld for a further 3-4 hours. The volume should not exceed 1 ml/100g body weight, except in the case of aqueous solutions where 2ml/100g body may be used[18].

CONCLUSION:

In acute toxicological study, the investigational product is administered at different dose levels, and the effect is observed for 14 days. All mortalities caused by the investigational product during the experimental period are recorded and morphological, biochemical, pathological, and histological changes in the dead animals are investigated. Acute toxicity study is involved in estimation of LD_{50} and also it is useful to determine the absolute dose given to the species in a row was multiplied by the factor given at intersection of the relevant row and column. OECD Guideline 420, OECD Guideline 423 and OECD Guideline 425 these methods are used to determine acute oral toxicity of chemical substance and herbal products.

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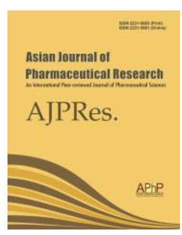

REVIEW ARTICLE

Acute Toxicity study of Synthesized drug and Herbal Product

Tanuja Yadav*, Sachin Rohane
Faculty of Pharmacy, Yashoda Technical Campus, Satara 415015, India.
*Corresponding Author E-mail: **tanujayadav711@gmail.com**

ABSTRACT:
Acute toxicity study describes the adverse effects of a substance that result either from a single exposure or from multiple exposures in a short period of time. Whenever an investigator administered a chemical substance or herbal drug to a biological system different types of interactions can occur and a series of dose-related responses result. In most cases these responses are desired and useful, but there are a number of other effects which are not advantageous. These may or may not be harmful to the patient. Acute toxicity study is involved in estimation of LD_{50}. Also it determines the therapeutic index i.e ratio between the lethal dose and the pharmacologically effective dose in the same strain and species. This article Review the methods so for utilized for the determination of acute toxicity.

KEYWORDS: Acute toxicity study, Therapeutic index, Biological system.

INTRODUCTION:
The amount of pharmacological substances, chemical and herbal drugs being used in the human community today have increased to almost an innumerable amount. These may be presented today in the form or as constituents of food substances medicines, beverages, otherindustrial and household products. However these chemicals or herbal drug may result in chronic toxicity in the living system when used over a long period of time or acute toxicity may also occur when large quantities capable of eliciting immediate toxic effect are used. These effects may be mild or severe depending upon nature of the substance.

Acute toxicity is defined as the unwanted effect that occurs either immediate or at a short time interval after a single or multiple administration of such substance within 24 hours. The unwanted effect is any effect is any effect that produces functional impairments in organs and/or biochemical lesion which could alter the functioning of the organism in general or individual organs [1-2].

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Toxicity is the fundamental science of poisons. The organization for Economic and Development (OECD) mentioned acute toxicity as the advance effects occurring within short time of oral administration. Phychochemical interactions of poisons leads to the injury or death of living tissues. Toxicology is like science and an art like medicine. It includes observational data gathering and data utilization to predict outcome of exposure in human and animals. Acute toxicity is distinguished from chronic toxicity, which describes the adverse health effects from repeated exposures, often at lower levels to a substance over a longer time period[3-4].

Acute toxicity (LD_{50}) test:
Acute systemic toxicity evaluates the adverse effects that occur following exposure of organisms to a single or multiple doses of a test substance within 24 hours by a known route (oral, dermal or inhalation). After administration, the test substance is absorbed and distributed to various parts of the body before it elicits systemic adverse effect. The regulatory body requires the acute toxicity test report for labelling and classification of substances for human use.

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The LD_{50} (median lethal dose) test was introduced in 1927 by J.W. Trevan to estimate the dose of a test substance that produces 50% death in a given species of animals. It is usually the first test conducted for every chemical before further toxicity tests are carried out. It is used for estimating the potential hazards of chemicals on humans. Although its major endpoint is death, non-lethal acute effect may occur as sign of toxicity depending on the chemical being tested.

Assessment of the acute toxic potential of substances is required to determine their adverse effects that might occur due to accidental or deliberate short-term exposure. Result from acute toxicity test serve as a guide in dosage selection for long term toxicity studies are well as other studies that involve the use of animals.

From the result of an acute toxicity test, a conclusioncan be made on the toxicity status of the substance. As depicted in table 1, substance with LD_{50} below 5 mg/kg are classified to be highly toxic while substance with LD_{50} above 15,000 mg/kg are termed relatively harmless [5-8].

Table 1: Classification of LD_{50} based on dose range.

LD_{50}	Classification
<5 mg/kg	Extremely toxic
5-50 mg/kg	Highly toxic
50-500 mg/kg	Moderately toxic
500-5000 mg/kg	Slightly toxic
5000-50000 mg/kg	Practically non-toxic
>15000 mg/kg	Relatively harmless

OECD Guideline for Acute Oral Toxicity:

OECD Guideline for the testing of chemicals are periodically reviewed in the light of scientific progress or changing assessment practices.The conventional acute oral toxicity test (formerly OECD Test Guideline 401)is the most heavily criticized test in terms of animal welfare and this concern was the driving force behind the development of three alternative tests for acute oral toxicity (Test Guideline 420,423,425).Anticipating the presence of validated alternatives, Member countries took the initiative to plan the deletion of Guideline 401[9-12].

A Nominated Expert Meeting (Rome 1998) and an Expert Consultation Meeting, (Arlington 1999) were convened to determine the acute oral toxicity data requirement needs of Member countries and to assess the capabilities of the alternatives to meet these needs. On the basis of these technical discussions, the 29th Joint Meeting concluded in June 1999 that not all data needs could be met by the alternatives (and not always by Guideline 401). The Joint Meeting decided that Guidelines 420,423 and 425 should be revised to meet regulatory needs of member of countries.

The revision of Guidelines 420, 423, and 425 was completed in 2000 following a second Expert Consultation Meeting (Paris 2000) and process of deletion of guideline 401 was started[13-15].

OECD Guideline 420:
Acute Oral Toxicity-Fixed Dose Procedure:

The method provides information on the hazardous properties and allows the substance to be ranked and classified to the Globally Harmonised System (GHS) for the classification of chemicals which cause acute toxicity.

Principle of the test:

Group of animals of a single are dosed in a stepwise procedure using thefixed doses of 5, 50, 300 and 2000 mg/kg (exceptionally an additional fixed dose of 5000 mg/kg may be considered). The initial dose level is selected on the basis of a sighting study as the dose expected to produce some signs of toxicity without causing severe toxic effects or mortality. Further groups of animals may be dosed at higher or lower fixed doses depending on the presence or absence of signs of toxicity or mortality. This procedure continues until the dose causing evident toxicity or no more than one death is identified or when no effect are seen at the highest dose or when death occur at the lowest dose.

Description of the method:

1. **Selection of animal species:**

The preferredrodent species is the rat. Normally females are used. When the test is conducted in males, adequate justification should be provided. Females should be nulliparous an non pregnant. Each animal, at the commencement of its dosing, should be between 8 and 12 weeks old and its weight should fall in an interval within ± 20 % of the mean weight of any previously dosed animals.

2. **Housing and feeding condition:**

The temperature of the experimental animal room should be 22 c^0. Although the relative humidity should be at least 30% and preferably not exceed 70%.Lighting should be artificial ,the sequence being 12 hours light ,12 hours dark . For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water.

3. **Preparation of animals:**

The animals are randomly selected, marked to permit individual identification and kept in their cages for at least 5 days prior to the start of dosing to allow for acclimatisation to the laboratory conditions.

4. **Preparation of doses:**

Test substances should be administer in a constant volume over the range of doses. In case of liquid end product or mixture is to be tested –use undiluted test

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substance i.e at a constant concentration .In either case,the maximum dose volume for administration must not exceeded .In rodents , the volume should not normally exceed 1ml /100g of body weight however in case of aqueous solution 2ml/100g body weight can be considered. For vehicles other than water the toxicological characteristics of the vehicle should be known.

Procedure:

1. Administration of doses:

By gavage using a stomach tube or suitable incubation canula(unusual circumstance –fraction of doses).Fasted prior to dosing. Weigh animals and administer the test substance. Withheld food for a further 3-4 hours in rats or 1-2 hours in mice (in case of fraction of doses).

2. Sighting study:

The purpose of the sighting study is to allow selection of the appropriate starting dose for the main study. The substance is administered to single animals in a sequential manner following the flow charts.The starting dose for the sighting study is selected from the fixed dose levels of 5,50,300 and 2000 mg/kg as a dose expected to produce evident toxicity. Also, evidence from in vivo and in vitro data from the same chemical and from structurally related chemicals. In the absence of such information, the starting dose will be 300 mg/ kg. At least 24 hours will be allowed between the dosing of each animal. Consideration of use of an additional upper fixed dose level of 5000 mg/kg. In cases where an animal tested at the lowest fixed dose level (5 mg/kg) in the sighting study dies, the normal procedure is to terminate the study and assign the substance to GHS category. For further confirmation use supplementary procedure.

3. Main study:

Numbers of animals and dose levels

Select dose from sighting study. Perform study on a total of five animals of one sex at each level including animals tested in sighting study. Time interval between dosing at each level depends on onset, duration and severity of toxic signs. Treatment of animals at the next dose should be delayed until one is confident of survival of the previously dosed animals. To check delayed Toxicity-A period of 3 or 4 days between dosing at each dose level is recommended. In case of inconclusive response time interval may be adjusted as appropriate. Fixed dose of 5000 mg/kg procedure outline in Annex 4.

4. Limit test:

Performed when information indicating that the test material is likely to be nontoxic i.e. having toxicity only above regulatory limit doses. How information about the toxicity of the test material can be gained?. Using the normal procedure test will be done [16].

OECD Guideline 423:

Acute Oral Toxicity-Acute Toxic Class Method:

The acute toxic class method is based on biometric evaluation with fixed doses, adequately separated to enable a substance to be ranked for classification purposes and hazard assessment. The method as adopted in 1996 was extensively validated in vivo against LD_{50} data obtain from the literature, both nationally and internationally.

Principle of the test:

It is the principle of the test that, based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step i.e.

- No further testing is needed,
- Dosing of three additional animals with the same dose.
- Dosing of three additional animals at the next higher or the next lower dose level.

Description of the method:

1. Selection of animal species:

The preferred rodent species is the rat. When the test is conducted in males adequate justification should provided. Healthy young adult animals of commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant. Each animal, at the commencement of its dosing, should be between 8 and 12 weeks old and its weight should fall in an interval with in ±20%of the mean weight of any previously dosed animals.

2. Housing and feeding conditions:

The temperature in the experimental animal room should be 22^0C. Although the relative humidity should be at least 30% and preferably not exceed 70%. Lighting should be artificial, the sequence being 12 hours light, 12hours dark for feeding conventional laboratory diets may be used with an unlimited supply of drinking water.

3. Preparation of animals:

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

4. Preparation of doses:

Test substances should be administer in a constant volume over the range of doses. In case of liquid end product or mixture is to be tested-use undiluted test substance i.e. at a constant concentration. In either case,

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the maximum dose volume for administration must not exceeded. In rodent, the volume should not normally exceed 1 ml/100 g of body weight however in case of aqueous solution 2 ml/100 g body weight can be considered. For vehicles other than water the toxicological characteristics of the vehicle should be known.

Procedure:

1. Administration of doses:

The test substance is administered in a single dose by gavage using a stomach tube or suitable intubation canula. Single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 hours. Animal should be fasted prior to dosing. Following the period of fasting, the animals should be weighed and the test substance administered. After the substance has been administered, food may be withheld for a further 3-4 hours in rats or 1-2 hours in mice.

2. Number of animals and dose levels:

Three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels 5, 50, 300 and 2000 mg/kg body weight. The starting dose level should be that which is most likely to produce mortality in some of the dosed animals. The flow charts of Annex 2 describe the procedure that should be followed for each of the starting doses.

When available information suggests that mortality is unlikely at the highest starting dose level, then a limit test should be conducted. When there is no information on a substance to be tested, for animal welfare reasons it is recommended to use the starting dose of 300 mg/kg body weight.

The time interval between treatment groups is determined by the onset, duration, and severity of toxic sighs. Treatment of animals at the next dose, should be delayed until one is confident of survival of the previously dosed animals. Exceptionally, and only when justified by specific regulatory needs, the use of additional upper dose level of 5000 mg/kg body weight may be considered.

3. Limit test:

The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic i.e. having toxicity only above regulatory limit doses. Information about the toxicity of the test material can be gained from knowledge about similar tested compounds or similar tested mixtures or products, taking into consideration the identity and percentage of components known to be of toxicological significance. In those situation where there is little or no information about its toxicity, or in which the test material is expected to be toxic, the main test should be performed.

A limit test at one dose level of 2000 mg/kg body weight may be carried out with six animals. Exceptioally a limit test at one dose level of 5000 mg/kg may be carried out with three animals. If test substance-related mortality is produced, further testing at the next lower level may need to be carried out [17].

OECD Guideline 425:

Acute Oral Toxicity: Up and Down procedure-

This test procedure is of principal value in minimising the number of animals required to estimate the acute oral toxicity of a chemical and in estimating a medium lethal dose. The medium lethal dose allows for comparison with historical data. In addition to the observation of mortality, LD_{50} allows the observation of signs of toxicity. The latter is useful for classification purpose and in the planning of additional toxicity tests.

Principle of the test:

Animals are dosed, one at a time, at 24 hours intervals. The first animal receives a dose at the level of the best estimate of the LD_{50}. Depending on the outcome for the previous animal, the dose for the text animal is adjusted up or down. If an animal survives, the dose for the next animal is increased; if it dies, the dose for the next animal is decreased. After reaching the reversal of the initial outcome i.e. the point where an increasing dose pattern is reversed by giving a smaller dose, four additional animals are dosed following the same UDP. The LD_{50} is calculated using the method of maximum likelihood.

Description of the method:

1. Selection of animal species:

The preferred rodent species is the rat. In the normal procedure female rats are used. When there is adequate information to infer that males are more sensitive, they should replace females in the test. The females should be nulliparous and non-pregnant. At the commencement of the study, the weight variation of the animals should be minimal and not exceed ±20% of the mean weight for each sex. The test animals should be characterised as to species, strain source, sex, weight and age.

2. Housing and feeding conditions:

The temperature in the experimental animal room should be 22^0 C. Although the relative humidity should be at least 30% and preferably not exceed 70%. Lighting should be artificial, the sequence being 12 hours light and 12 hours dark. For feeding conventional laboratory diets may be used with an unlimited supply of drinking water.

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3. Preparation of animals:
The animals are uniquely identified and kept in their cages for at least five days prior to dosing for acclimatisation to the laboratory conditions. Animals demonstrating signs of spontaneous disease or abnormality prior the start of the study are eliminated from the study.

4. Preparation of doses:
When necessary, the test substance is dissolved or suspended in a suitable vehicle. It is recommended that, whenever possible, the use of an aqueous solution or suspension be considered first, followed by consideration of a solution or emulsion in oil and then by possible solution in other vehicle. For vehicles other than water, toxicity of the vehicle must be known.

Procedure:

1. Full test:
Individual animals are dosed in sequence at 24 h intervals, one at time and then observed for a minimum of 24 h. However, the time intervals between dosing should not be fixed rigidly and may be adjusted as appropriate, in case of delayed mortality. The first animal is dosed at the toxicologist's best estimate of the LD_{50}. If the animal survives, the second animal receives a higher dose, unless the limit dose was used as the starting dose. If the first animal dies or appears moribund the second animal receives a lower dose. Moribund state is characterised by symptoms such as shallow, laboured or irregular respiration, muscular weakness or tremors, absence of voluntary response to external stimuli, cyanosis and coma, Criteria for making the decision to humanely kill moribund and severely suffering animals are the subject of a separate Guidance Document. Animals killed for humane reasons are considered in the same way as animals that died on test.

For selecting the starting dose, all available information should be used, including information on structure-activity relationships. When the information suggests that mortality is unlikely then a limit test should be conducted. When there is no information on the substance to be tested, for animal welfare reasons it is recommended to use the starting dose of 200 or 500 mg/kg body weight. The dose for each successive animal is adjusted up or down, depending on the outcome of the previous animal. If feasible, a dose progression factor of 1.3 is used. Other factors may be used, if justified. After reaching the reversal of the initial direction, four additional animals are dosed using the same UDP. This is the end of the normal test.

2. Limit test:
Doses should not exceed 2000 mg/kg which is considered the upper limit dose. When the first animal is dosed with the upper limit dose and survives, the second animal receives the same dose. When total of three animals have been dosed with the limit dose and no deaths have occurred, then three animals of the other sex should be tested at the limit dose level. If there is again no lethality, the test can be terminated.

3. Optional testing:
Information from one sex may be adequate toxicity. However, if found desirable, comparability of response in the other sex can be evaluated by administering to generally not more than 3 animals, dose above and below the estimated LD_{50}. The point intermediate between doses where responses change can be taken as an appropriate estimate of the lethal dose.

4. Administration of doses:
The test substance is administered in a single dose by gavage, using an oral dosing needle or rubberised tubing. The animal should be fasted prior to dosing by withholding food overnight. Fasted body weight of each rat is determined and the dose is calculated according to the body weight. After dosing food may be withheld for a further 3-4 hours. The volume should not exceed 1 ml/100g body weight, except in the case of aqueous solutions where 2ml/100g body may be used[18].

CONCLUSION:

In acute toxicological study, the investigational product is administered at different dose levels, and the effect is observed for 14 days. All mortalities caused by the investigational product during the experimental period are recorded and morphological, biochemical, pathological, and histological changes in the dead animals are investigated. Acute toxicity study is involved in estimation of LD_{50} and also it is useful to determine the absolute dose given to the species in a row was multiplied by the factor given at intersection of the relevant row and column. OECD Guideline 420, OECD Guideline 423 and OECD Guideline 425 these methods are used to determine acute oral toxicity of chemical substance and herbal products.

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Skeletal muscle relaxant effect of *Bacopa monnieri* (L.) natural and micropropagated plant extracts

Firoj A Tamboli[1*], Vinod D Rangari[2], Harinath N More[1], Vijay H Kutwade[3], Vivek M Patil[3], Ashish T Thorat[4]
[1] Department of Pharmacognosy, Bharati Vidyapeeth College of Pharmacy, Kolhapur, Maharashtra, India
[2] SLT Institute of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya (Central University), Koni, Bilaspur, Chhattisgarh, India
[3] Department of Pharmaceutical Quality Assurance, Bharati Vidyapeeth College of Pharmacy, Kolhapur, Maharashtra, India
[4] Yashoda Technical Campus, Faculty of Pharmacy, Satara, Maharashtra, India

Abstract
This research work was carrying out to evaluate the natural and micropropagated *Bacopa monnieri* plant extract for skeletal muscle relaxant activity. The skeletal muscle relaxant activity of the extracts was evaluated using Rota rod test. The skeletal muscle relaxant activity of control and treated mice were recorded and statistically correlated among the control, standard and the test drugs. For muscle relaxant property total fall off time for standard and control group was recorded.

Keywords: *Bacopa monnieri*, natural plant, micropropagated plants, skeletal muscle relaxant activity, rotarod test

Introduction

Bacopa monnieri is also known as Brahmi, belongs to family Scrophulariaceae. In the traditional system of medicine the plant is used for number of activities like laxative, carminative, digestive, anti-inflammatory, anti-convulsant, bronchodilator, febrifuge, and nervine tonic [1-6]. The drug mainly contain bacosides which is triterpenoid saponins. Hersaponin, one of its active principle, is reported to have a sedative effect.[7-9] The plant shows cardioprotective and hepatoprotective effects. The plant is an aphrodisiac, effective in treating scabies and syphilis, and purifies the blood, having proven useful for diarrheas and pyresis [10-11].
The plant tissue culture techniques are used for the investigations of the secondary metabolites. Already 2000 plants have been reported to be regenerated through the plant tissue culture. It has also been shown that many of such plants can produce secondary metabolites in culture. Tissue culture (often called micropropagation) is a special type of asexual propagation where a very small part of tissue (shoot apex, leaf section, or even an individual cell) is excised (cut-out) and placed in aseptic culture in a test tube, petri-dish or tissue culture container containing a special culture medium. Tissue culture technology has been known as an effective tool to propagate valuable medicinal plants. Therefore now plant tissue culture has been included as an important tool under biotechnology [12-17].
This research work was carried out to conduct skeletal muscle relaxant of natural and micropropagated plant of *Bacopa monnieri*

Material and Methods

Collection and authentication of plant

Bacopa monnieri was collected from field of Jawaharlal Nehru Ayurvedic Medicinal Plant Garden and Herbarium Kothrud, Pune in the month of February, 1998 and authenticate the plant from same institute. After collection plant material was washed thoroughly with water and kept for drying in the sunlight for 4-5 days. After drying, the plant material was broken into very small pieces and then passed through crusher mill, to obtain coarse powder. The powder was passed though sieve no.12.

Micropropagated tissue culture plant

Shoot tips and nodel segments of B. monnieri L were cultured on Murashige and Skoogs (M. S.) basal medium supplemented with different concentration of BAP and IAA. [12].

Materials

Chemicals and Pharmaceuticals: Chlorpromazine, Tween 80 Instrument and equipment: Rotarod, Animals: Swiss albino mice Others: Syringe, injection needles, weighing balance.

Preparation of drug solution

Ethanolic extract of *B. monnieri* natural (BMN) plant, Ethanolic extract of *B. monnieri* micropropagated (BMM) plant and Ethanolic extract of *B. Monnieri* Standard (BMS) were prepared by dissolving required amount of BMN ethanolic extract in distilled water. A drop of tween 80 was used to prepare uniform suspension. Chlorpromazine (2 mg/kg, ip): It was prepared by dissolving 0.2 mg of chlorpromazine in 1 ml of distilled water.

Pharmacological Screening

Rotarod Test (Muscle relaxant activity)

This test was used in particular to screen myorelaxant activity of the drug. Motor weakness is detected by failure of mice to cling to the rotating rod. In this test only those mice were selected which were capable of remaining on the rotating rod normally for more than 2 minutes. The rod was maintained at the speed of 12 rpm and the mice were kept on the rod facing its back towards the side of observer. Fall time has been used to assess the ability of control and treated mice, individually in each group at the interval of 30 minutes for the period of two and a half-hours. In this experiment, the mice were divided in to 5 groups each groups carrying 6 mice. The first group was served as control and was treated with vehicle

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(tween 80, 0.5% v/v, ip). The second BMN plant ethanolic extract, third BMM plant ethanolic extract and Fourth group BMS ethanolic extract (100 mg/kg ip) of each respectively and last group receiving chlorpromazine (2 mg/kg, ip).[18]

Result

Rotarod Test (Muscle relaxant activity)

Table 1: Effects of ethanol extracts of *B. Monnieri* natural, micropropagated and standard on muscle grip in albino mice by Rotarod test

Treatment (Dose and route)	Rotarod Test (Muscle relaxant activity) in min.					
	30	60	90	120	180	240
Control (tween 80, 0.5%, ip.)	0	0	0	0	0	0
BMN extract (100mg/kg, ip)	0	0	0	0	0	0
BMM extract (100 mg/kg, ip)	0	0	0	0	0	0
BMS extract (100 mg/kg, ip)	0	0	0	0	0	0
Chlorpromazine (2 mg/kg, ip)	3	5	5	4	1	0

Control: tween 80, 0.5% v/v, ip
BMN: *Bacopa Monnieri* Natural plant extract, 100 mg/kg, ip.
BMM: *Bacopa Monnieri* Micropropagated plant extract, 100 mg/kg, ip.
BMS: *Bacopa Monnieri* Standard extract, 100 mg/kg, ip &
CPZ: Chlorpromazine 2 mg/kg ip.

Discussion

The ethanolic extract of BMN, BMM and BMS were evaluated for muscle relaxant activity in mice using rotarod test. The extract failed to show muscle relaxant activity (Table 1).Present pharmacological study with ethanolic extract of BMN, BMM and BMS showed muscle relaxant activity. Such study with natural plan of *B. monnieri* have been confirmed by many worker but the similar study on the micropropagated plants needs to be evaluated**.**

Conclusion

The pharmacological profile indicates that the extract failed to show muscle relaxant activity.

Conflict of interest

The authors declare no conflict of interest.

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RESEARCH ARTICLE

RP-HPLC Method Development and Validation of Tadalafil in Tablet Dosage form

Chavan Pooja Ajit[1], Shelar Reshma Dattatraya[1], Shelake Pallavi Ramchandra[1], Avinash Mahadeo Bhagwat[1], Dr. Ajit Bhiva Ekal[2]
[1]YSPM's Yashoda Technical Campus, Faculty of Pharmacy (B. Pharm), Satara
[2]Managing Director of Insta Vision Laboratory and Service, Satara
*Corresponding Author E-mail: **chavanp978@mail.com, reshmadshelar10@gmail, pallavishelakestr@gmail**

ABSTRACT:
Tadalafil is a phosphodiesterase 5 inhibitor accustomed to treat dysfunction, benign prostatic hyperplasia, and pulmonary arterial hypertension. Tadalafil is an orally administered drug and it's marketed worldwide under the name Cialis. Analytical method development and validation play important roles within the discovery development and manufacture of pharmaceuticals. These methods is to make sure the identity, purity, potency, and performance of drug product. The bulk of the analytical development effort goes into validating a stability indicating HPLC–method. Analytical method development could be a process of proving that the developed chromatography method is suitable for its intended use within the development and manufacturing of the pharmaceutical drug substance and drug product. All analytical methods that are intended to be used for analyzing any clinical samples will have to be validated. The target of the strategy validation is to demonstrate that the strategy is suitable for its intended purpose because it is stated in ICH guidelines.

KEYWORDS: Tadalafil, Phosphodiesterase 5 Inhibitor, Erectile dysfunction, High performance liquid chromatography, Validation.

INTRODUCTION:

Male erectile dysfunction has been defined as the persistent inability to attain and maintain an erection adequate to permit satisfactory sexual performance. Although erectile dysfunction is regarded as a benign disorder, it has a medical and social impact due to its high prevalence, costs and implications for the quality of life for many men and their partners.

A recent review concludes that the prevalence of erectile dysfunction of all degrees is 52% in men 40 to 70 years old, with the incidence increasing with advancing age. Normal erectile function requires the coordination of psychological, hormonal, neurological, vascular and anatomic factors. Alteration of any of these factors is sufficient to cause erectile dysfunction.[1]

Tadalafil is reversible phosphodiesterase type 5 (PDE5) inhibitor approved for the treatment of erectile dysfunction (ED). As a category PDE5 inhibitors (including sildenafil and vardenafil), enhance erectile response to sexual stimulation by increasing penile blood flow. The duration of action of Tadalafil is longer than sildenafil or vardenafil.[2]

Tadalafil (CIALIS) is an orally administered PDE5 inhibitor has been developed for a treatment for erectile dysfunction. When sexual stimulation causes the local release of nitric oxide gas, which plays a central role within the vasodilation of erectile tissues by stimulating guanylyl cyclase activity, consequently raising intracellular concentrations of cyclic guanosine monophosphate (cGMP) and relaxing vascular smooth muscle. This leads in smooth muscle relaxation and inflow of blood into the penile tissues, thereby producing an erection. Thus tadalafil is indicated for the treatment of male erectile dysfunction. Tadalafil has no effect within the absence of sexual stimuli.[1]

Tadalafil is a phosphodiesterase 5 inhibitor accustomed treats erectile dysfunction, benign prostatic hyperplasia, and pulmonary arterial hypertension. Tadalafil is practically insoluble in water. It does not possess any ionisable groups within the pH range of 1-11 and, subsequently, doesn't demonstrate any changes in solubility in aqueous buffers in this range. It is freely soluble only in solvents like as dimethylsulfoxide and dimethylformamide.[1]

This molecule has 2 chiral centers and thus four different stereoisomers are also found. The molecule obtained in the process described is within the RR form. Crystallization studies show that Tadalafil doesn't exhibit polymorphism.[1]

Chemistry:

1. Synonyms: Adcirca, Cialis, GF196960, HSDB7370, IC351, ICOS351, Tadalafil, Tadalafil Lilly, UNII-742SXX0ICT
2. IUPUC:(6R,12aR)-6-(1,3-benzodioxol-5-yl)-2-methyl-2,3,6,7,12,12a-hexahydropyrazino[1',2':1,6]pyrido[3,4-b]indole-1,4-dione
3. Formula: C22H19N3O4
4. Molar mass: 389.411 g·mol−1 [3]

Figure 1: Chemical Structure of Tadalafil

This study is to develop an easy and accurate RP-HPLC method for the estimation of Tadalafil in tablet dosage form. The method validation is to demonstrate that the strategy is suitable for its intended purpose because it is stated in ICH guidelines. The strategy was validated for linearity, precision, accuracy, specificity, and limit of detection, limit of quantification, robustness and system suitability.[4]

MATERIAL AND METHOD:

Material:

Tadalafil pure drug sample obtain from Tadacip 20 tablet which is manufactured at Cipla Ltd, India. HPLC grade Acetonitrile was procured from E. Merck Ltd, India. SQ grade Potassium Hydrogen Orthophosphate was purchased from Fisher Scientific. AR grade Orthophosphoric Acid was purchased from Rankem. Milli-Q water was used throughout the experiment. Tadacip 20 tablets were purchased from local pharmacy.

Instrumentation:

Analysis was performed on a Summit HPLC chromatographic system, Low Pressure Quaternary Gradient Dionex manufacturer equipped with ASI-100 Automated sample injector, LPG-4 HPLC Pump, programmable variable wavelength PDA-3000 detector. Chromatographic separation was achieved by Agilent eclipse C_{18} column (4.6 x 250mm, 5um). The HPLC system was equipped with "Chromeleon 6.8 SR 11" software to acquire and process the data. Peak purity was checked the PDA detector.[5]

METHODOLOGY:

Standard solutions:

Weigh accurately and transfer about 40mg Tadalafil standard in 200ml volumetric flask. Add about 150ml of Mobile phase. Sonicate for 5 minutes. Allow the solution to attend room temperature and dilute up to mark with mobile phase.

Mobile phase:

Phosphate Buffer pH 4.0: 1.360gm. of Potassium Di-hydrogen Orthophosphate dissolved and diluted in 1000 ml water. Adjust the pH to 4.0 with dilute ortho phosphoric acid. Phosphate buffer pH 4.0: Acetonitrile (50:50). Mix, Sonicate and filter through 0.45 micron nylon filter paper.

Chromatographic Conditions:

An isocratic condition HPLC analysis was performed an Agilent Eclips C18 (150 x 4.6mm, 5um) maintained at conditions (30°C). Chromatographic separation was achieved with mobile phase and mixture of at flow rate of 1.0ml/min and injection volume of 20µl and also the run time is 6 min. the Tadacip 20 was scanned under conditions and from the spectra maxima of 284nm was observed.

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Assay of formulation:
Procedure:
Weigh 20 Tablet at a time. Calculate Average weight. Crush Mix to make uniform and perform assay from it.

Sample Preparation:
Weigh and transfer sample powder containing about 40 mg of Tadalafil in 200ml volumetric flask. Add about 150ml of mobile phase. Sonicate for 20 minutes. Allow to attend room temperature. Dilute up to mark with mobile phase. Filter with whatman 41 or equivalent filter paper.

Inject blank, standard and Sample preparation as sequence given below,

(1) Blank (2) Standard preparation (5 replicates) (3) Blank (4) Sample preparation (5) Standard preparation (Bracketing standard)

Perform calculation for system suitability parameters –% RSD of retention time and area of standard replicates, cumulative %RSD of retention time and area of replicate standards with bracketing standard.

System suitability criteria:
1. %RSD for retention time of replicates of standard preparation should not be more than 1.0%
2. %RSD for area of replicates of standard preparation should not be more than 2.0%
3. Theoretical plates for all the standard injections should not be less than 2000. Report Theoretical plate of first replicate of standard preparation.
4. Tailing factor for all the standard injections should not be less than 2.0. Report tailing factor of first replicate of standard preparation.
5. Cumulative %RSD for retention time of replicates of standard preparation with retention time of bracketing standard should not be more than 1.0 %
6. Cumulative %RSD for area of replicates of standard preparation with area of bracketing standard should not be more than 2.0%

If system suitability found within limit, perform calculations for content of Tadalafil Tablet by using formula, Calculate Content of Tadalafil (mg/Tablet) for sample preparations independently by using formula described below.

$$= \frac{\text{(Area of Tadalafil in sample preparation)}}{\text{(Avg. area of Tadalafil Standard preparation)}} \times \frac{W1\ (mg)}{200} \times \frac{200}{W2\ (g)} \times \frac{P1}{100} \times W3\ (gm.)$$

Where,
W1 = Weight of Tadalafil standard taken for preparation of standard preparation (in mg)
W2 = Weight of Tadalafil sample taken for preparation of sample preparation (in g)
W3 = Average weight of Tablet in gm.
W = Weight of sample in gm.
P1 = % Purity of Tadalafil Standard

Acceptance Criteria:
Each film coated tablet content:
Tadalafil per tablet = 18.00mg to 22.00mg

% Assay = 90.00% to 110.00%

Method validation:
A. Specificity and system suitability:
- Experimental design:
1. Inject mobile phase to observe interference at interested peaks.
2. Prepare and inject std preparation individually (as per final conc. Of mixed std preparation).

- **Acceptance criteria:**
1. There should not be interference of any peak at interested peaks.
2. System suitability parameter should meet and assay for tadalafil 20mg should be within limit.

B. Precision
a) Repeatability:
- Experimental design:
  Perform assay in six individual replicates of sample. Perform % RSD of assay.
- Acceptance criteria:
  % RSD for assays should be within less than 2%

b) Intermediate precision:
- Experimental design:
1. Perform assay in six individual replicates of sample by other person on other day with other set of chemicals.
2. Perform %RSD of six sample preparations.
3. Also calculate % variation of average assay values obtained via repeatability and intermediate precision.

- **Acceptance criteria:**
1. Assay for all samples should be within limit.
2. % RSD for assays for intermediate preparation should be less than 2%.
3. % variation of average assay values obtained via repeatability and intermediate precision should be less than 3%.

C. Accuracy:
- **Experimental design:**
1. Prepare samples in triplicate by taking weight equivalent to 80%, 100% and 120% of working levels and perform analysis.
2. Calculate % recovery by factors amount added vs.

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amount recovered.
3. Calculate %RSD of recovery at each level for triplicate preparations.

- **Acceptance criteria:**
1. % recoveries of individual preparation should be 98 to 102%.
2. % RSD at each level should not be more than 3%

D. Linearity and range:
- Experimental design:
1. Prepare standard preparations at each 80%, 100% and 120% of working level and inject them.
2. Determine co-relation coefficient by plotting linearity graph. Calculate % y intercept

- **Acceptance criteria:**
1. Graph should be linear and co-relation coefficient should be not less than 0.999
2. % y intercept should be within ±2%

E. LOD AND LOQ
- **Experimental design:**
1. 5ppm solution to be prepared and signal to noise ratio is determined.
2. LOD is determined by preparing diluted solutions with signal to noise ratio about 3.
3. Level of concentration at which peak got detected repeatedly but not necessary to be precise is LOD.
4. LOQ is determined by preparing diluted solutions with signal to noise ratio above 3 and about 10.
5. Level of concentration at which peak got detected with precise area (%RSD NMT 2%) is LOQ.

- **Acceptance criteria:**
1. For LOD Single to noise ratio is about 3.
2. For LOQ Single to noise ratio is about 10.
3. For LOQ, %RSD for replicate injections should be less than 2 %

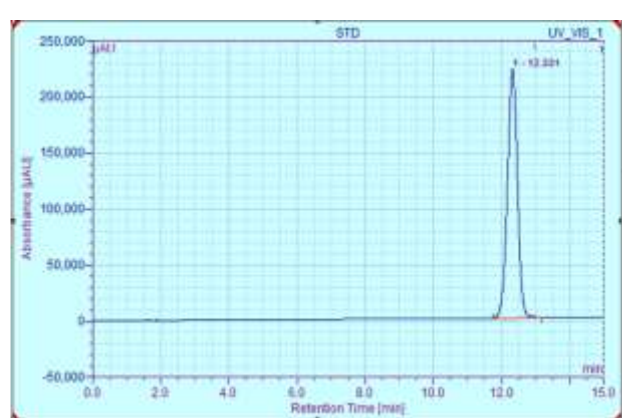
F. Robustness:
- Experimental design:
1. Perform analysis of sample by changing flow rate as 0.8 and 1.2ml per minutes.
2. Perform analysis of sample by changing wavelength 282 nm and 286nm.
3. Calculate % cumulative RSD of assay obtained at repeatability and by changing parameters.
- Acceptance criteria:
1. % cumulative RSD of assay obtained at repeatability and by changing parameters should not be more than 3 %

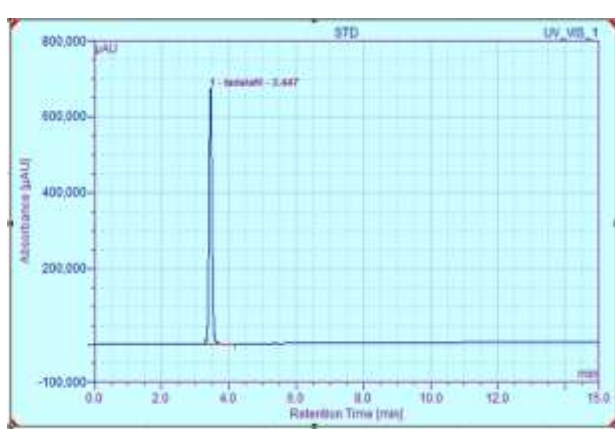
Non-conformance:
Specify the non-conformance (if any) observed during method validation.

RESULT AND DISCUSSION:

Method development and optimization:
In order to optimize the LC conditions for the estimation of Tadalafil in tablet the following trials were performed. Initially a mobile phase consisting of 50mM Potassium Di-hydrogen Orthophosphate (pH 5.0): Acetonitrile (80:20 %v/v) at a flow rate of 1.0mL/min was used on an Agilent Eclipse C18 column (250 x 4.6) column at ambient temperature using mobile phase as diluent, Tadalafil did not elute under these conditions,

In the next trial, same column was employed but the mobile phase was changed to mobile phase consisting of 10 mM Potassium Di-hydrogen Orthophosphate (pH 5.0): Acetonitrile (70:30% v/v) at a flow rate of 1.0 mL/min was used on an Agilent Eclipse C18 column (250 x 4.6) column at ambient temperature.





Tadalafil eluate with at 12.331 with theoretical plates 4234 (limit NLT 2000 and Tailing factor 1.58 (NMT2) which well within the limit but as retention time is more and will time consuming during analysis therefore focusing on reduction of retention time drastically. pH of buffer solution reduced with dilute Orthophosphoric acid

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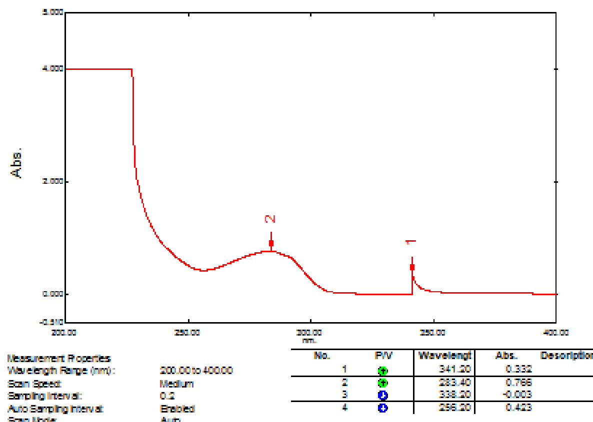
solution. Organic Solvent content in mobile phase composition increased and also temperature of column increased.

Tadalafil eluate with at 3.447 with theoretical plates 3431 (limit NLT 2000 and Tailing factor 1.55 (NMT 2) which well within the limit. Thus further injection of same std is done and to conclude precision. Corresponding area, RT and system suitability parameters observed.

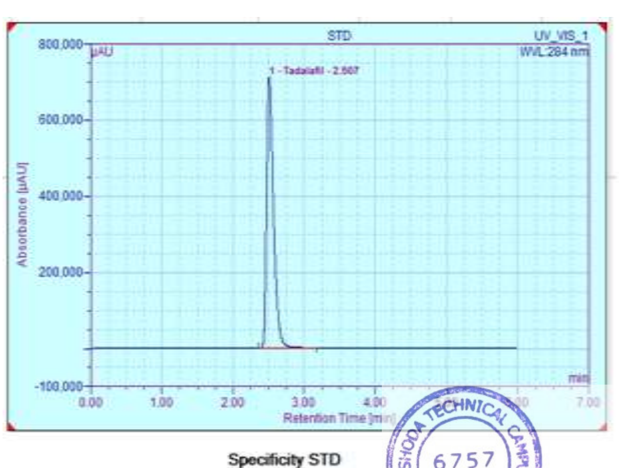
Lambda max is determined before conducting next trial and found 284nm.Next trial conducted with mobile phase consisting of 10mM Potassium Di-hydrogen Orthophosphate (pH 4.0): Acetonitrile (50:50% v/v) at a flow rate of 1.0mL/min was used on an Agilent Eclipse C18 column (250 x 4.6) column at 30°C.



Method validation

A. Specificity And System Suitability:

Specificity demonstrated by observing interference of mobile phase (Diluent). System suitability parameters (% RSD of area, Retention time, Theoretical Plates and Tailing factor) demonstrated by injecting standard preparation in replicate.



Specificity STD

Table no1: Specificity and System Suitability

Std Inj No	Retention Time	Area	Theoretical Plates	Tailing Factor
1	2.507	80482.079	3436	1.59
2	2.507	81838.312	3359	1.59
3	2.508	80682.928	3412	1.58
5	2.507	80378.131	3398	1.57
5	2.509	80181.986	3422	1.58
% RSD	0.04 (Limit NMT 1 %)	0.81 (Limit NMT 2 %)	Limit: NLT 2000	Limit: NMT 2

No interference observed from Mobile phase (Diluent).

Conclusion – Method found specific and capable to achieve System suitability.

B. PRECISION:

A. Repeatability:

The repeatability was demonstrated by preparing the standard solution at 200ppm concentration and six independent consecutive sample preparations at 200 ppm. System suitability found within limit. Relative standard deviation of assay value for six preparations found within 2 %.

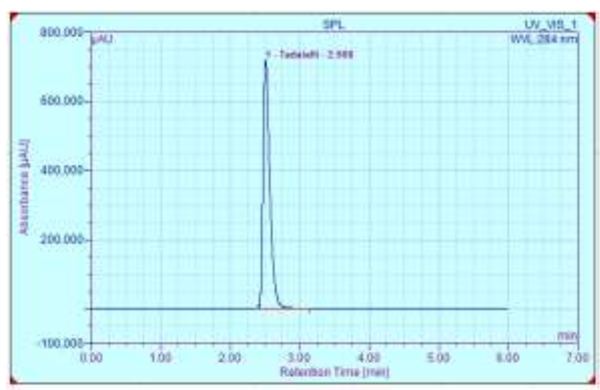


Table no 2: Repeatability Suitability System

Std Inj No	Retention Time	Area	Theoretical Plates	Tailing Factor
1	2.507	80550.869	3436	1.59
2	2.508	80234.452	3392	1.58
3	2.507	81223.21	3414	1.57
4	2.505	80877.456	3433	1.58
5	2.508	80281.714	3390	1.58
% RSD	0.05 (Limit NMT 1 %)	0.52 (Limit NMT 2 %)	Limit: NLT 2000	Limit: NMT 2

Average std area = 80633.5402

Sample	RT	Area	% Assay
1	2.508	81050.398	101.35
2	2.507	81124.947	101.16
3	2.507	81038.768	101.38
4	2.508	81098.332	100.71
5	2.509	81168.295	101.06
6	2.509	81068.679	100.84
% RSD (NMT 2 %)			**0.27**

B. Intermediate Preparation:

The Intermediate Precision was demonstrated by preparing the standard solution at 200ppm concentration

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and six independent consecutive sample preparations at 200ppm. By other person on other day with other set of chemicals. System suitability found within limit. Relative standard deviation of assay value for six preparations found within 2%. % variation of average assay values obtained via repeatability and intermediate precision found within 3%

Table no 3: Intermediate Preparation System Suitability

Std Inj No	Retention Time	Area	Theoretical Plates	Tailing Factor
1	2.507	84508.997	3369	1.58
2	2.507	84122.505	3278	1.57
3	2.504	83453.121	3245	1.56
5	2.505	84467.887	3314	1.56
5	2.504	83567.886	3243	1.57
% RSD	0.06 (Limit NMT 1 %)	0.59 (Limit NMT 2 %)	Limit: NLT 2000	Limit: NMT 2

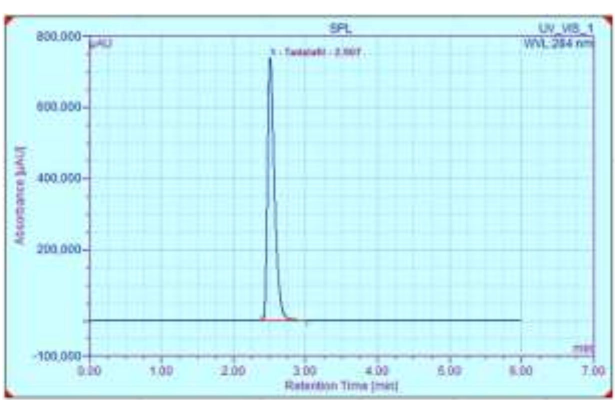
Average std area = 84024.0792

Sample	RT	Area	% Assay
1	2.507	84235.965	100.47
2	2.507	84156.235	100.52
3	2.509	84578.691	100.92
4	2.509	84269.523	101.28
5	2.508	84638.419	100.84
6	2.507	84398.651	100.86
% RSD (NMT 2 %)			**0.29**
% RSD with repeatability (NMT 3 %)			**0.30**

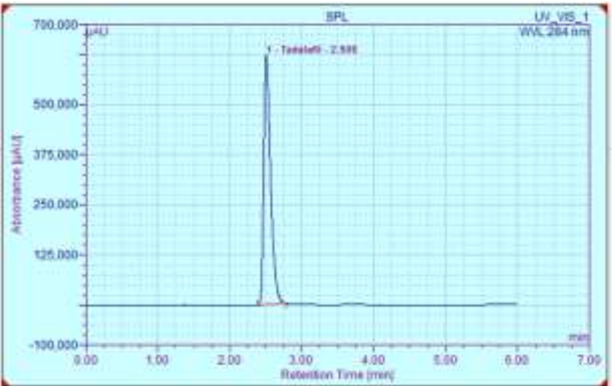
Conclusion – Method found Precise.

C. ACCURACY:

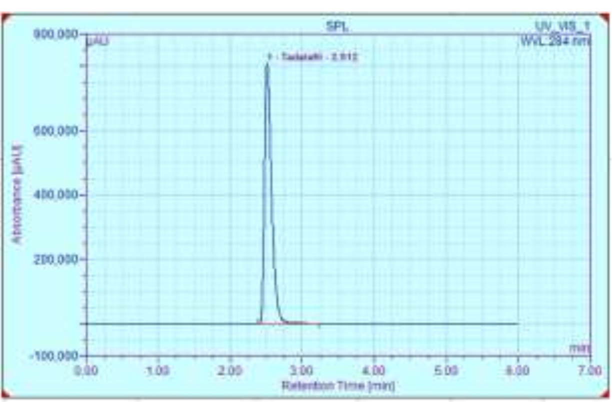
To determine the accuracy of the method, recovery studies were carried out in triplicate by using different concentrations of pure drug in the pre analyzed samples with 3 different concentrations of sample that consists of 80 %, 100 % and 120 % of the pure drug. The accuracy was expressed as the percentage analytes recovered.



Accuracy SPL 100 %



Accuracy SPL 80 %



Accuracy SPL 120 %

Table no 4: Accuracy

Std Inj No	Retention Time	Area	Theoretical Plates	Tailing Factor
1	2.506	84122.127	3312	1.55
2	2.505	84613.560	3214	1.55
3	2.504	83948.654	3344	1.54
4	2.508	84026.334	3370	1.56
5	2.509	84386.329	3351	1.55
% RSD	0.08 (Limit NMT 1 %)	0.33 (Limit NMT 2 %)	Limit: NLT 2000	Limit: NMT 2

Sample	% Recovery	Average % recovery	% RSD
80 %	99.66	99.54	0.36
80 %	99.13		
80 %	99.82		
100 %	98.94	99.39	0.41
100 %	99.73		
100 %	99.51		
120 %	99.15	99.25	0.55
120 %	98.76		
120 %	99.84		
Limit		**Limit 98 to 102 %**	**NMT 3 %**

Conclusion – Method found Accurate.

D. LINEARITY AND RANGE:

From the standard stock solution, the various dilutions of Tadalafil in the concentration of 160.0, 200.0, 240.0 ppm three level standard solutions of each were prepared. The solutions were injected using 20 µL injection volumes in

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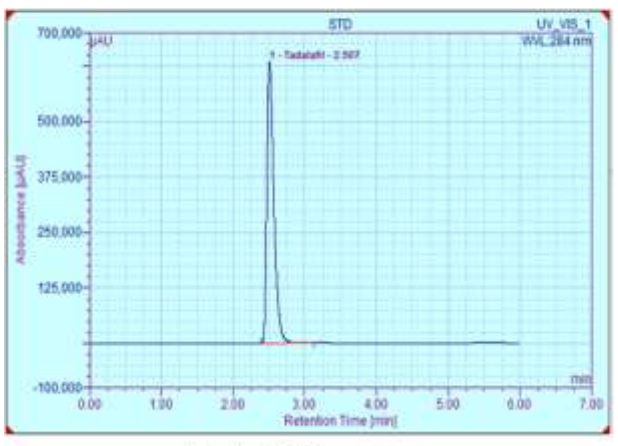
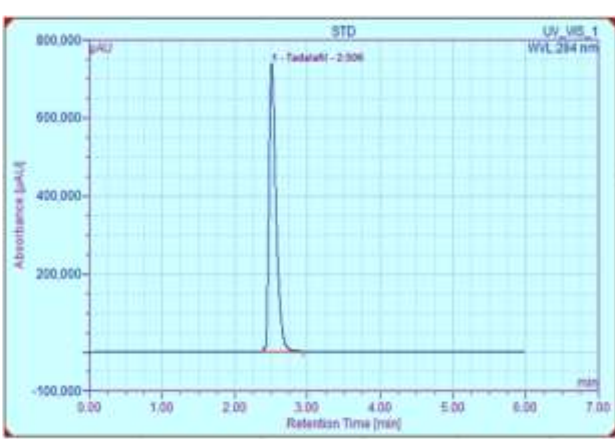
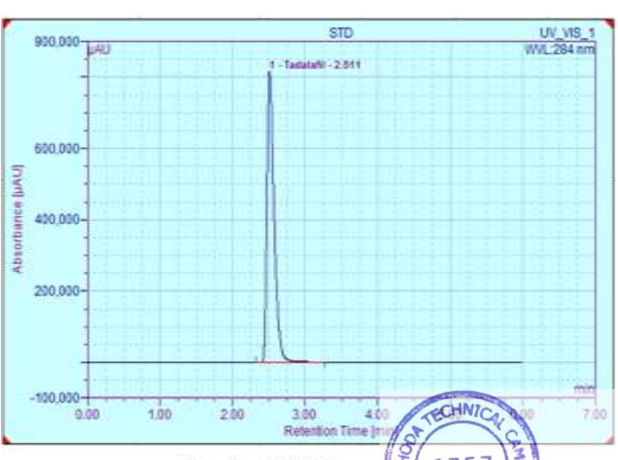
to the chromatographic system at the flow rate of 1.0 ml/min and the effluents were monitored at 284nm, chromatograms were recorded. Calibration curve of Tadalafil was obtained by plotting the peak area ratio versus the applied concentrations of Tadalafil by using average of each sample. The linear correlation coefficient (R2) was found to be 1.000 and %y intercept is 0.0035%



Linearity std 80 %



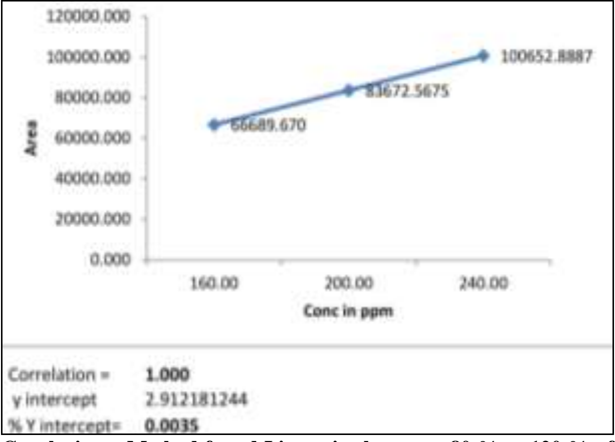
Linearity std 100 %



Linearity std 120 %

Table No 5: Linearity and Range

Sr. No.	Conc. ppm	Area	Average
1	160.0	66710.597	66689.670
2	160.0	66638.432	
3	160.0	66719.981	
4	200.00	83676.153	83672.5675
5	200.00	83698.982	
6	200.00	83642.568	
7	240.00	100407.985	100652.8887
8	240.00	100598.458	
9	240.00	100952.223	



Conclusion – Method found Linear in the range 80 % to 120 % of working level

E. Limit of Detection and Limit of Quantitation (LOD and LOQ):

The limit of detection and limit of quantification means the lowest concentration of analytes in the sample are detected and quantified. LOD and LOQ was found as listed below

Table no 6: LOD and LOQ

Parameter	Obtained value
LOD	0.00035 ppm
LOQ	0.00523 ppm

F. ROBUSTNESS:

Robustness of the method was determined by intentionally changing some operating conditions such as flow rate and wavelength. The flow rate as per the developed method is 1.0ml/min. It has been purposely changed to 0.8ml/min and 1.2ml/min and the chromatogram was developed as well as the wavelength of developed method is 284nm. It has been purposely changed to 282nm and 286nm and the chromatogram was developed.

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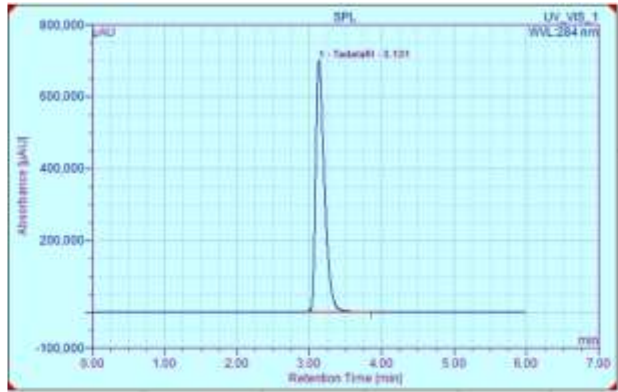
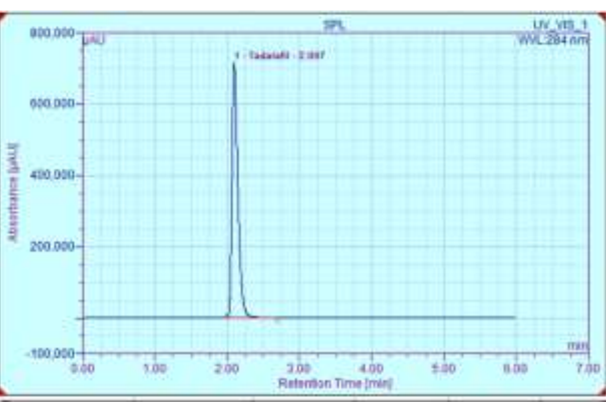
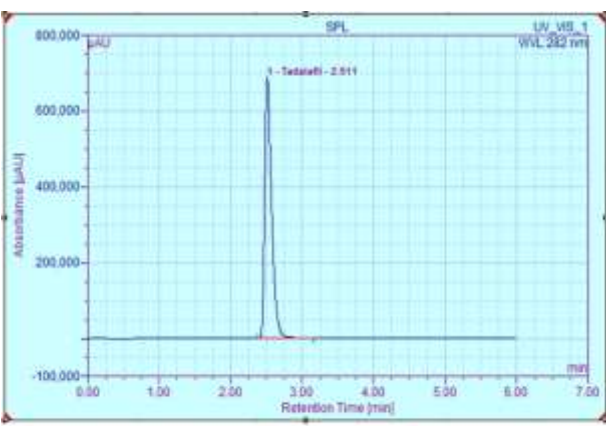


SPL 0.8 ml / min



SPl 1.2 ml / min



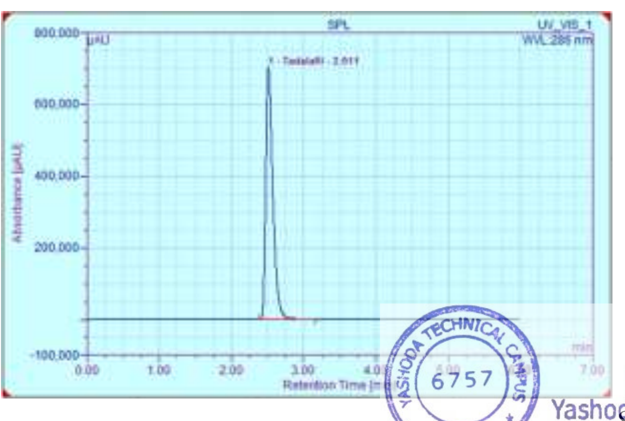


Table No 7: Robustness system suitability flow rate = 0.8 ml

Std Inj No	Retention Time	Area	Theoretical Plates	Tailing Factor
1	3.128	100735.480	3388	1.59
2	3.126	100123.563	3321	1.58
3	3.124	99131.118	3339	1.57
4	3.125	99790.264	3350	1.59
5	3.128	99520.003	3297	1.59
% RSD	0.06 (Limit NMT 1 %)	0.61 (Limit NMT 2 %)	Limit: NLT 2000	Limit: NMT 2

Parameter	Condition	RT	Area	% Assay	% Cumulative RSD with Repeatability
Change in flow rate	0.8 ml /min	3.131	99300.247	100.11	0.38

Table no 8: Robustness system suitability flow rate = 1.2 ml /min

Std Inj No	Retention Time	Area	Theoretical Plates	Tailing Factor
1	2.098	68319.994	3335	1.56
2	2.097	67222.965	3334	1.55
3	2.098	68210.003	3330	1.54
5	2.099	68427.675	3323	1.56
5	2.096	69276.998	3245	1.56
% RSD	0.05 (Limit NMT 1 %)	1.07 (Limit NMT 2 %)	Limit: NLT 2000	Limit: NMT 2

Parameter	Condition	RT	Area	% Assay	% Cumulative RSD with Repeatability
Change in flow rate	1.2 ml /min	2.097	68411.243	101.43	0.24

Table no 9: Robustness system suitability detection wavelength = 282 nm

Std Inj No	Retention Time	Area	Theoretical Plates	Tailing Factor
1	2.097	80130.345	3287	1.58
2	2.096	80023.776	3186	1.56
3	2.093	80211.543	3233	1.57
5	2.095	81235.867	3295	1.58
5	2.097	80425.971	3231	1.58
% RSD	0.08 (Limit NMT 1 %)	0.61 (Limit NMT 2 %)	Limit: NLT 2000	Limit: NMT 2

Parameter	Condition	RT	Area	% Assay	% Cumulative RSD with Repeatability
Change in wavelength	282nm	2.096	77812.828	100.11	0.38

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Table no 10: Robustness system suitability detection wavelength = 286 nm

Std Inj No	Retention Time	Area	Theoretical Plates	Tailing Factor
1	2.097	79145.885	3212	1.56
2	2.095	78230.870	3198	1.55
3	2.092	79431.114	3243	1.54
5	2.094	79786.240	3230	1.57
5	2.096	79555.476	3211	1.55
% RSD	0.09 (Limit NMT 1 %)	0.76 (Limit NMT 2 %)	Limit: NLT 2000	Limit: NMT 2

Parameter	Condition	RT	Area	% Assay	% Cumulative RSD with Repeatability
Change in wavelength	286nm	2.097	80160.9116	100.78	0.23

% Cumulative RSD of % assay observed for changing parameters calculated and found within limit i.e. below 3 %.

Conclusion – Method found Robust.

Non-conformance: Specify the non-conformance (if any) observed during method validation.

CONCLUSION:

The proposed RP-HPLC method was simple, sensitive, precise and accurate for determination of Tadalafil n tablet dosage form. The results obtained for all validated parameters were within the limits; hence the proposed method can be easily applied for the quantification of Tadalafil in routine quality control pharmaceutical laboratories. The analytical method used for determination of assay of Tadalafil Tablet is within acceptance criteria for the analytical parameters such as Specificity and system suitability, Linearity and Range, Precision, Accuracy and Robustness. Hence method stands validated.

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SDC-PC BASED SOLID SEDDS OF BCS CLASS III DRUG

Aneri V. Adsul[1]*, Bharatee P. Chaudhari[2] and Vivekkumar K. Redasani[3]

[1]Student, Department of Pharmaceutics, YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Satara. Maharashtra, India.

[2]Assistant Professor, Department of Pharmaceutics, YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Satara. Maharashtra, India.

[3]Principal, Department of Pharmaceutics, YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Satara. Maharashtra, India.

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***Corresponding Author**
Aneri V. Adsul
Student, Department of Pharmaceutics, YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Satara. Maharashtra, India.

ABSTRACT

Aim: The purpose of this research is to develop, optimise, and analyse losartan potassium loaded solid SEDDS using a newly synthesised surfactant. Losartan potassium is an angiotensin II receptor blocker that belongs to the BCS class III of antihypertensive drugs. Solid SEDDS were used to increase the permeability, oral bioavailability, and first pass metabolism of losartan potassium, which had a low permeability and oral bioavailability. **Material and methods:** As a surfactant, SDC-PC was synthesized. The solubility of the API in various oils, surfactants, and co-surfactants was investigated, and oleic was chosen as the oil phase, SDC-PC as the surfactant, and PEG 400 as a co-surfactant for formulation. A pseudo-ternary phase diagram was created to get the ideal emulsification area. SEDDS liquid was prepared and tested. Following an evaluation, it was discovered that LP4 was stable and optimum. **Result and discussion:** Aerosil 200 was used as a carrier to convert the formulation into solid SEDDS. The formulations were compared to LOSAR®, as marketed product. On in-vitro drug release, optimised batch LP4 was shown to have similar drug release to the marketed formulation.

KEYWORDS: Losartan potassium, SEDDS, Surfactant, Solid dosage form, Pseudo-ternary phase diagram.

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INTRODUCTION

Losartan potassium (LP) is a non-peptide angiotensin II receptor antagonist (Type AT1) that is orally active and undergoes substantial first-pass metabolism by the cytochrome P450 enzyme, with 14 % of the dose converting to an active metabolite.[1,2] LP is a class III drug that comes in the form of a white to off-white free-flowing crystalline powder with a log P value of 5.37, a half-life of about 2 hours, and a systemic bioavailability of about 33%.[1,2,3] The drug is used once or twice a day as a 25 mg tablet, with total daily doses ranging from 25 to 100 mg.[2] In diabetic patients, LP provides beneficial pressure control, lowering the risk of stroke and the progression of renal disease to the terminal stage.[4] LP binds to plasma proteins extensively and can cause gastrointestinal problems, neutropenia, active hepatotoxicity, migraines, and pancreatitis.[5] To reduce the frequency of dose and adverse effects of LP, sustained drug delivery is required to prolong the drug release, and self-emulsifying drug delivery can be employed to achieve this.[3]

Self emulsifying formulations are defined as isotropic mixtures of natural or synthetic oil, liquid or solid surfactant or one or more hydrophilic solvents and co-solvent or surfactants.[6] Upon mild agitation followed by dilution in aq-media, such as GI fluids, these system can form oil-in-water(o/w) emulsion(10-100nm).[7] SEDDS' physical qualities, as well as the chemical structures of its constituents, were found to be important determinants of application and tolerability.[8] These fine microemulsion droplets have the benefit of providing the drug in a dissolved form with a large interfacial surface area for drug absorption, resulting in improved, uniform, and repeatable bioavailability.[9] The oral bioavailability of both hydrophobic and hydrophilic drugs can be improved by increasing membrane fluidity to facilitate transcellular absorption, opening tight junctions to allow paracellular transport, inhibiting cytochrome P450 as isoenzyme in the intestinal region, and inhibiting efflux pumps such as P-glycoprotein.[10] Using a self-emulsifying drug delivery system to improve oral bioavailability of BCS class III medicines has a ton of potential. These systems have been found to be useful in the formulation of first-pass metabolism medications as well as orally delivered pharmaceuticals that obtain access to the systemic circulation through direct absorption into the intestinal lymphatic system.[11]

Following mechanisms are implicated for the improvement of permeability.

- Gastric retention time – The oil in SEDDS can decrease the gastric empting time.

- Lymphatic transport – The oil in SEDDS may enhance the lymphatic transport and the bioavailability of highly lipophilic drugs by promoting their association with chylomicrons in the enterocytes and avoiding hepatic metabolic pathway.
- Intestinal protein efflux – oil and non-ionic surfactants in SEDDS may reversibly inhibit P-glycoprotein and the multidrug resistance related proteins -2 efflux transport or increases the transcellular permeability.
- First pass metabolism – SEDDS may inhibit the action of cytochrome P450 enzyme which metabolizes drug in intestinal wall.[12]

Because liquid SEDDS have disadvantages including instability, low convenience, production processes, interaction during filling in capsule shells, and storage temperature, solid SEDDS were developed. When compared to precursor liquid SEDDS, solidification provides a number of advantages, which can be summarised as better drug solubility and dissolution, improved safety, controlled or sustained drug release, and industrial and commercial benefits.[13]

The aim of our present study to develop, optimize and evaluate losartan potassium loaded solid SEDDS using synthesized surfactant. As surfactant plays important role to reduce surface tension between two different phases. The vesicular based system act as reservoir for the control release of a number of active drug including antibiotics, corticosteroids. They also act as permeation enhancer in systemic absorption.[14]

MATERIALS AND METHODS

Losartan potassium was obtained from YARROW CHEM PRODUCTS, Mumbai. Oleic acid was obtained from S. D. Lab chemical centre, Mumbai. Aerosil 200 was obtained from Gangwal chemical, Mumbai. Stearoyl chloride was obtained from Dolphin pharmacy instrument Pvt. LTD, Mumbai. Sulfanilamide, acetone, n-hexane, ethyl acetate, caster oil, iso propyl myristate, PEG 400, Tween 80. all the chemicals and solvents used in this work belonged to analytical grade.

Synthesis of surfactat

N-((4-sulfamoylphenyl) carbamothioyl) stearamide (SDC-PC) was synthesized by taking stearoyl chloride 0.67 mL (2 mmol) and KSCN 194 mg (2 mmol) in 50 mL round bottom flask equipped with reflux condenser in 20 mL acetone. The resulting mixture was then stirred for 2 hours at 60 °C. After 2 hours, Sulfanilamide 344 mg (2 mmol) was added and

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refluxed the reaction mixture for further 18 hours (Scheme 1). The progress of the reaction was monitored periodically using TLC in ethyl acetate and n-hexane (3:7, v/v) solvent system.[14]

Evaluation of synthesized surfactant

% Practical yield

Percent practical yield was calculated by following formula:

% practical yield = $\text{Practical yield} \div \text{Therotical yield} \times 100$

Melting point

Melting point apparatus was used to determine the melting point of SDC-PC. In the melting point apparatus, a little amount of SDC-PC was inserted in one end of a closed capillary and the temperature at which the drug melted was recorded and compared to previous research.

ATR-FTIR Spectroscopy

The ATR-FTIR spectrum of surfactant was collected using the ATR-FTIR instrument BRUKER-Alpha 100508. A small amount of drug was collected and applied directly to the ATR diamond. A pressure pump was used to push the medication. The spectrum was obtained by combining 24 scans across a range of 4000-400cm.[15] The precise wavelength of light was partially absorbed by the sample, and at least one was reflected off the internal surface in contact with the sample. The ATR-FTIR spectrum depicts percent transmittance in terms of light wavelength (cm). For all interaction, the spectrum of the sample was acquired and compared to the spectrum of the pure drug.[16]

Critical Micelles Concentration (CMC) Determination

The CMC of all the newly synthesized surfactants were determined spectrophotometrically using UV-visible spectrophotometer (Shimadzu, UV-1800, Japan). Surfactant were dissolved in ethanol in different concentrations i.e. 0.01–0.1 mM read spectrophotometrically. A plot for each concentration versus its absorption was made and then straight lines were drawn on the values. The critical micelle concentration was the point where two straight lines intersect each other on this graph of concentration vs absorption.[14]

Solubility study

Solubility studies in various oils, surfactants, and co-solvents were conducted in order to determine the optimal SEDDS excipients with good solubilizing capacity for losartan

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potassium.[17] In a glass vail containing an excess of Losartan potassium, one (1) ml of each of the selected oil, surfactant, and co-surfactant sample was added (50-70 mg). To achieve equilibrium, the vails were shaken for 72 hours on an orbital shaker at 40 ° C. After that, aliquots of the supernatants were taken and filtered using a 0.45m membrane filter. Filtration with a 0.45m membrane filter separated the unmixed drug. The filtered sample was centrifuged for 15 minutes at 3000 rpm.

Construction of ternary phase diagram

Phase diagram provide useful platform for delineating the area of microemulsion. Ternary phase diagrams of oil, surfactant/co-surfactant (Smix) and water were developed using the water titration method.[19] Surfactant and co-surfactant were mixed up with six different (Km) weight ratios 1:9 to 9:1. For each phase diagram oil to specific Smix ratio was mixed in different proportion from 0.5:4.5 to 4.5:0.5. Nine different proportions are 0.5:4.5, 4:1, 3.5:1.5, 3:2, 2.5:2.5, 2:3, 1.5:3.5, 4:1 and 4.5:0.5. This made to maximum ratios were covered for the study to delineate the boundaries of phase precisely formed in the phase diagram. A transparent and homogenous mixture of oil/Smix was formed by using magnetic stirring. Then each mixture was titrated with drop wise addition under gentle agitation until the required clarity and flow ability was achieved. The point at which system become turbid, these points were recorded. Corresponding to these points calculate the % w/w combination of oil, surfactant and co-surfactant. Using these points phase diagram was constructed to determine the boundaries of microemulsion reason.[20] The phase diagram constructed using CHEMIX school software version 7.0.

Preparation of liquid SEDDS

By dissolving the amount of Losartan potassium as shown in the ternary phase diagram, a number of SEDDS formulations were created. The oil phase (125 mg) was placed in a vial, and the drug (25 mg of LP) was added straight to this vial and combined using a vortex mixer. To make an isotropic mixture, a sufficient amount of Smix was added to the oil-drug mixture and vortexed, followed by homogenization for 10 minutes. The formulation was checked for turbidity and phase separation before being stored at room temperature until further use.[21]

Evaluation of liquid SEDDS

Thermodynamic stability study

Heating cooling cycle (Freeze-thaw cycle): Six cycles of heating at 45˚C (incubator) and cooling at 4˚C (refrigerator) was conducted for not less than 48hrs at each temperature.

Centrifugation test: Those formulations which passed the heating cooling cycle test then subjected to centrifugation test at 3500 rpm for 30 min. Those formulations that did not show any sign of phase separations, which are most thermodynamically stable.[22]

Dispersibility test

The efficiency of self-micro emulsifying drug delivery system was evaluated by the dispersibility test. Dipersibility study was performed by adding each formulation in 500 ml of distilled water at 37˚C ± 0.5˚C. A standard stainless steel dissolution paddle rotating at 50 rpm provided gentle agitation. The in vitro performance of the formulation was visually evaluated using the following grading system.

- **Grade A:** Rapidly forming emulsion having a clear or bluish appearance (within 1 min)
- **Grade B:** Rapidly forming slightly less clear emulsion, having a bluish white appearance.
- **Grade C:** Fine milky emulsion was formed (Within 2 min).
- **Grade D:** Dull, grayish white emulsion having slightly oily appearance (Longer than 2 min).
- **Grade E:** Formula exhibiting either poor or minimal emulsification with large oil globules present on the surface.[16]

Self emulsification time

A standard USP dissolution apparatus type II was used to test the self-emulsification efficiency of SEDDS. In 900 ml of 0.1N HCI kept at 37 °C, a quantity equivalent to 25mg of each formula's microemulsion was added. A typical stainless steel dissolving paddle moving at 75 rpm provides agitation. The rate of emulsification and final appearance of the microemulsion were visually analysed for the created formulae. These investigations were carried out in order to better replicate the state of the stomach following oral ingestion. With respect to time, the tendency to emulsify spontaneously and the progression of emulsion droplets were observed.[23]

Globules size measurement and PDI

In a beaker, SEDDS formulation (1 ml) was diluted with 100 ml deionized water and constantly stirred with a glass rod. The microemulsion that resulted was then tested for globule size and PDI. Dynamic light scattering with particle size apparatus was used to determine the globule size and PDI of the resulting micrommalsion (Malvern Zetasizer, Ver. 7.12, serial Number: MAL 1098084, UK). The particle (droplet) size was measured at equilibrium. The lowest droplet size values that are more stable, isotropic, and transparent oil/water (o/w) dispersions that have a higher absorption rate potential.[24]

Zeta potential

Zeta potential is the electric potential in the interfacial double layer. Zeta potential is a key indicator of stability. It is indicating the electrostatic repulsion and congregation in oily droplets. The electrostatic repulsion of emulsion droplets plays an important role for assessment of stability of the system High electrostatic repulsion droplets prevent coagulation or flocculation on to fine emulsion droplets into larger oily globules.[25] Zeta potential determined by Zetasizer was monitored at 25°C at a scattering angle 173 (Malvern Zetasizer. Ver. 7.12, serial Number: MAL 1098084. UK)

% Transmittance

The percent clarity of the prepared samples was assessed to demonstrate the formulation's transparency. Using a UV-spectrophotometer and distilled water as a blank, the % transmittance of the system is determined at 650 nm wavelength. If the percent transmittance of a formulation is greater than 99 percent, the formulation is transparent.[21]

Drug content

The drug content was determined by dissolving SEDDS formulation equivalent to 10 mg drug in 50 ml of methanol and mixed well with shaking for two to three times 0.1 ml of this solution was diluted with fresh methanol, and drug content was determined spectrophotometrically (Shimadza 1800, Japan) at 233nm.[26]

Preparation of solid sedds

By using an adsorption approach, liquid SEDDS were turned into free-flowing powders, resulting in a more uniform drug release profile. Drops of liquid SEDDS were added to aerosil 200 in 1:0.25, 1:0.5, 1:1, 1:1.5, and 1:2 ratios, and the mixture was stirred for 5 minutes in a mortar pestle. To ensure a consistent dispersion of the formulation, the mixture

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was homogenised with a glass rod after each addition. The final mass was passed through mesh no. 120 (0.125mm), dried at room temperature, and stored in desiccators until further examination.[16,27]

Evaluation of solid sedds

Flow properties

Bulk density, tapped density, Hausner's ratio, Carr's index, angle of repose was evaluated.

Reconstitution properties of solid SEDDS

Effect of dilution on solid SEDDS

The property of quick emulsification was noticed after 100 mg S-SEDDS was correctly weighed and added to 100 ml distilled water in a beaker at 37°C and gently stirred with a magnetic stirrer at 100 rpm. The tendency to produce an emulsion was determined as follows:

- Good - If emulsification occurs in <1 min with clear or transparent emulsion.
- Bad – If emulsion if less clear or transparent[16]

In vitro dissolution test

The drug dissolution profile was studied using the USP dissolution apparatus II (Electrolab, Mumbai). Dissolution tests were performed in 900 ml of 0.01 N HCI (pH 2.0) at 37 0.5 °C with 75 rpm stirring. 10 mg of LP and formulation batches were introduced to the dissolution medium, and 5 ml samples were withdrawn after 10, 20, 30, 40, 50, and 60 minutes, and replaced with 5 ml fresh 0.5 percent Polysorbate 20 in 0.01 N HCL each time. The solutions were immediately filtered through a 0.45 um membrane filter, diluted, and UV-spectrophotometrically measured at 233nm.[28]

Drug and excipient interaction

ATR-FTIR spectra of pure LP, Aerosil 200, physical mixtires and Solid SEDDS formulations were recorded by ATR-FTIR Spectrometer (ALPHA 100508 BRUKER. US) to illustrate the promising interactions among the excipients used in the formulation. The spectrum was scanned over the wave number range of 4000-400 cm^{-1}[16]

RESULT AND DISCUSSION

% Practical yield

% Practical yield of synthesized surfactant was found to be 84.32%.

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Melting point

Melting point of synthesized surfactant was found to be 135˚C

Fourier transforms infrared (FTIR) spectroscopy

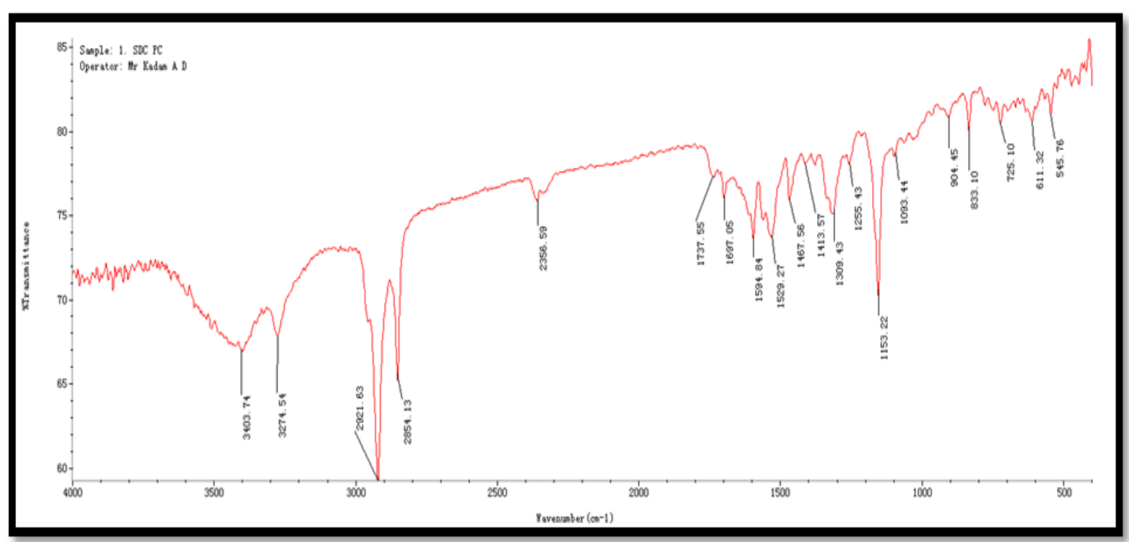
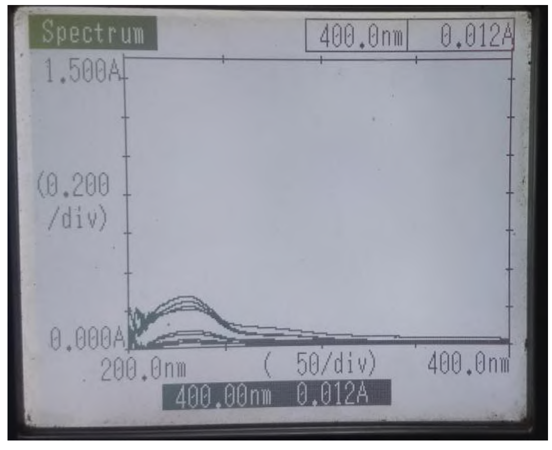


Fig. 1: ATR-FTIR spectrum of SDC-PC.

ATR-FRIR spectrum of SDC-PC is given in above figure. The spectrum shows NH_2 asymmetric at 3403.74 cm^{-1}, NH at 3274.54 cm^{-1}, CH_2 asymmetric at 2921.63 cm^{-1}, CH_2 symmetric at 2854.13 cm^{-1}, C=C aromatic at 1529.27 cm^{-1}, C-N at 1309.43 cm^{-1}, C-S at 1153.22 cm^{-1}. All characteristic peak of surfactant found in reported range.

Critical micelles concentration

The critical micelles concentration is an important phenomenon for scientist and researchers in the field of drug delivery. The micellization is the property of non ionic surfactant and polymers. No-ionic surfactant when exposed to the water forms aggregations called as micelles. When surfactants are added to a solvent, they are dispersed in the solution. By increasing the concentration of surfactant i the medium, at CMC they form micelles. CMC can be found by plotting graph of suitable physical property as a function of surfactant concentration. The CMC values of synthesized surfactant were determined by plotting the absorbance at λ_{max} against the concentration of each surfactant. The surfactant SDC-PC was read ranging from 0.01-0.1 µg/ml concentration and its CMC was calculated as 0.06mM.



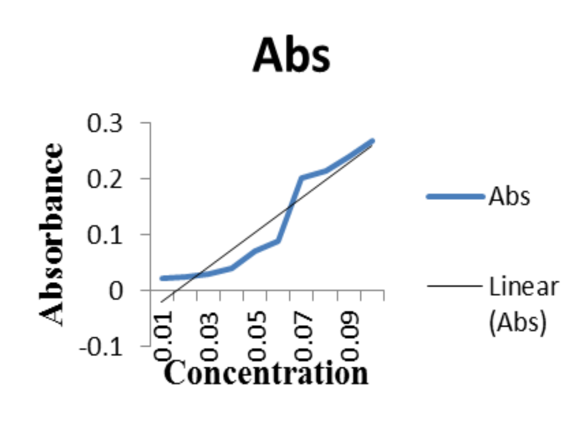


Fig 2: UV-visible spectra and CMC of SDC-PC.

Solubility study

After performing solubility study, the drug was found to be more soluble in oleic acid (oil), SDC-PC (surfactant), PEG400 (co-surfactant) results are shown in fig.

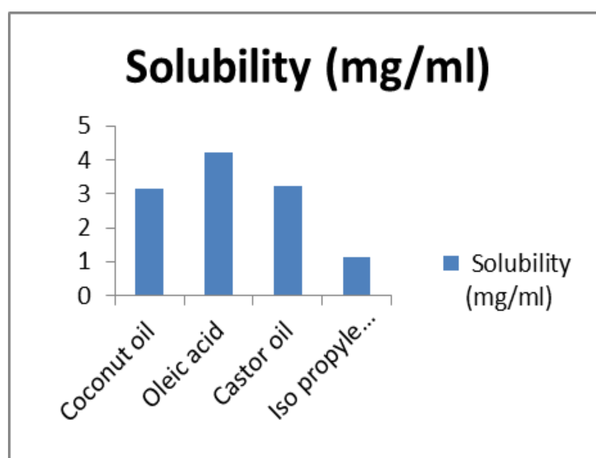


Fig. 3: solubility in various oils.

Solubility mg/ml

6 5 4 3 2 1 0

Tween 80 Tween 20 SDC-PC Span 80 Span 85

Solubility mg/ml

Fig. 4: Solubility in various surfactant.

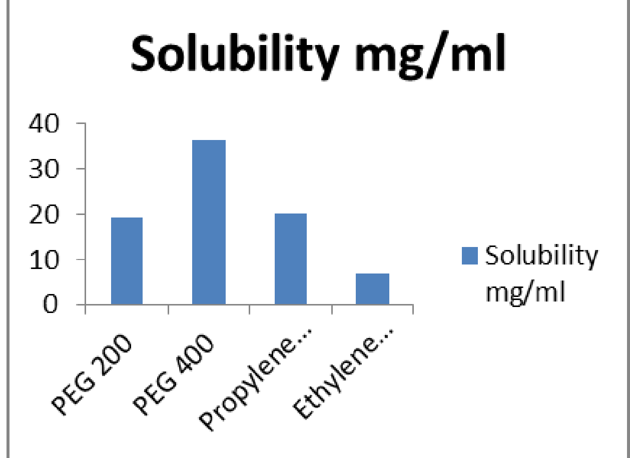


Fig. 5: Solubility in various co-surfactant.

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Construction of ternary phase diagram

The pseudo-ternary phase diagram is useful to determine the suitable combination of oil, surfactant and co-surfactant concentration in the formulation to form the nano-emulsion. After selection of oil, surfactant and co-surfactant based on the solubility study, the pseudo-ternary phase diagrams containing a fixed ratio of surfactant and co-surfactant (Smix) were constructed. The ternary phase diagram of the system containing SDC-PC: PEG 400 with the ratio of 1:9 formed with the wider emulsifying region in the presence of oleic acid as oil. It is clear from Fig.7 that the emulsifying region increases with an increase in the amount of surfactant mixture concentration in the system. Several reports explained this phenomenon of a decrease in the mean droplet size as an outcome of an increase in the concentration of surfactant and vice versa. The reduced droplet size with a high concentration of surfactant mechanism may be supported with the following statements:

- Stabilization of the oil droplet occurs with reduction in the interfacial tension between oil and water phase at a high concentration of surfactant.
- Enhancement of water penetration into oil in the presence of high surfactant which causes the release of oil droplets in aqueous phase

The addition of the drug did not affect the self-emulsifying region significantly.

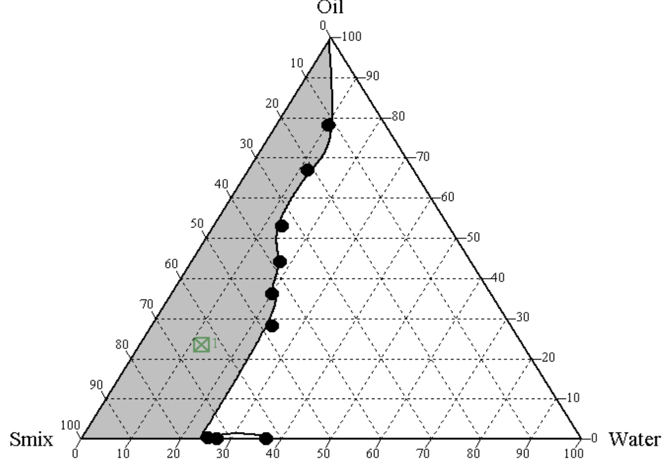

Fig. 7: Ternary phase diagram (Smix 1:9).

Preparation of liquid SEDDS

A total of four (4) formulations LP1 to LP4 were successfully prepared with their respective composition as shown in table 1.

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Table 1: Formulation table of Liquid SEDDS.

Batch code	Drug (mg)	Oil (%)	$Smix_1$ (%)	$Smix_2$ (%)	Weight of batch (%)
LP1	25	70	-	30	100
LP2	25	60	-	40	100
LP3	25	50	-	50	100
LP4	25	40	-	60	100

Thermodynamic stability study

The thermodynamic studies have always helped determine the kinetic stability of the formulation. The main criteria of microemulsion for pass this test is not to show any indication of phase separation, creaming, cracking or coalescence. All the prepared formulations had passed the thermodynamic study test, with no signs of phase separation and precipitation of drugs. This indicates that the prepared formulations were stable against the maintained storage conditions.

Dispersibility test

The dispersibility of microemulsion shows Grade A of all formulations showed a rapidly forming emulsion having a clear or bluish appearance (within 1 min).

Table 2: Dispersibility grades and self emulsification time.

Sr. No.	Batch	Dispersibility Grade	Self emulsification time (sec)
1	LP1	A	54
2	LP2	A	48
3	LP3	A	45
4	LP4	A	44

Self emusification time study

The emulsification time of all batches was found to be in the range of 44 to 54 seconds as shown in Table 2. The batch LP4 showed very short emulsification time. The determination of self emification time for the assesment of microemulsion spread or scatters in GIT medium. Smaller the size of particle faster the lease and shows the efficiency of formulation.

Globule size, PDI and Zeta potential

Globule size of emulsion plays important role in absorption and also in stability. Globule size of optimized formulation was observed 836.7 nm (Fig.8) which is within the range of nano-emulsion (500-1000nm) and polydispersity index (PDI) was observed 0.466. Zeta potential of

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optimized batch was observed -0.329 mV (Fig.9) negative potential around particles shows improved lymphatic uptake of system.

Table No.: Globule size, PDI and zeta potential of all batches.

Batch	Globule size	PDI	Zeta potential
LP1	583.8	0.975	0.527
LP2	1671	0.040	0.0608
LP3	974.6	0.604	-0.0518
LP4	836.7	0.466	-0.329

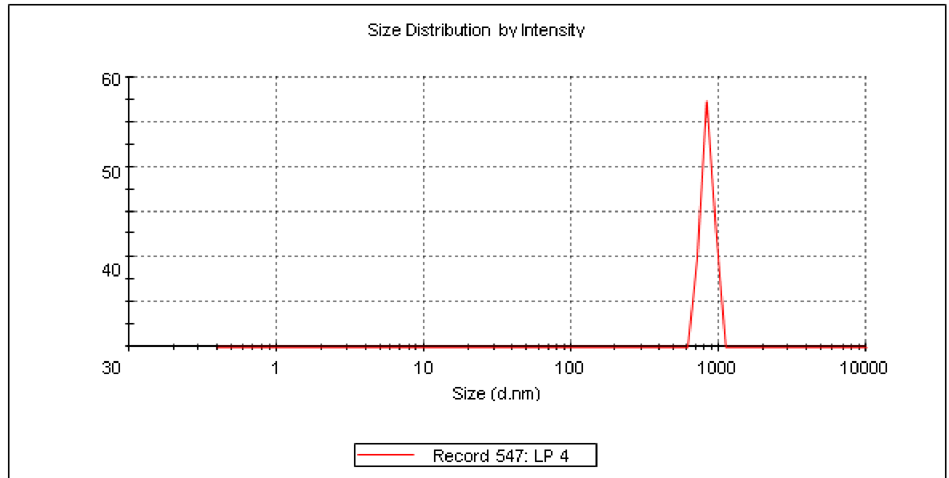

Fig. 8: Globule size distribution graph of optimized batch.

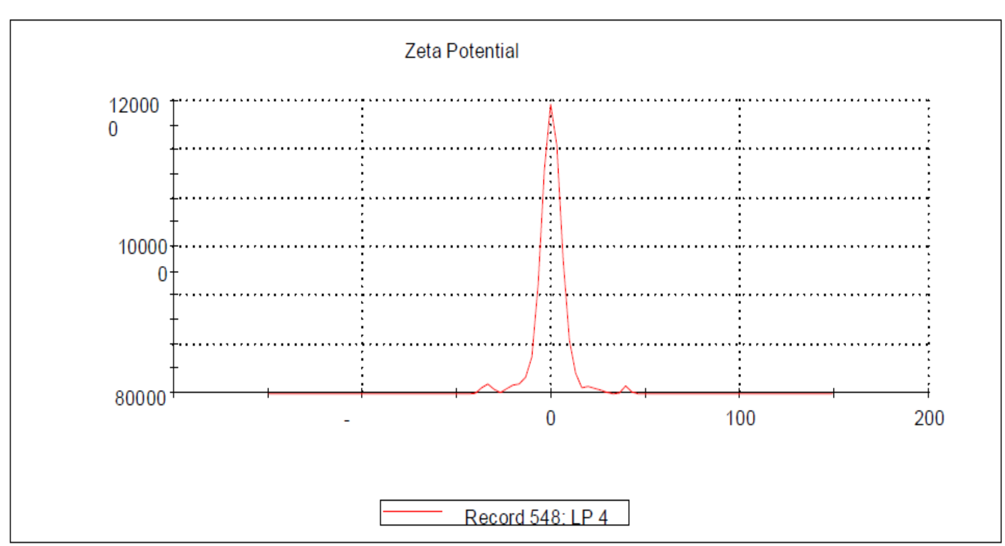

Fig. 9: Zeta potential of optimized batch.

% Transmittance measurement

Percent transmittance was evaluated for proving the transparency of formulation. A value closer to 100% confirms, the transparency of the formulation and indicates large surface area

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for drug release. Percent transmittance of formulation was found to be in the range of 100% to 93.2 % and tabulated in Table 3.

Table 3: % transmittance measurement.

Sr. No.	Batch	% Transmittance
1	LP1	96.1
2	LP2	98.3
3	LP3	99.0
4	LP4	100.3

Drug content

The drug content of the prepared losartan potassium loaded SEDDS was determined to evaluate the uniformity of dose in the formulation. The drug content in different prepared batches is listed in table 29. Drug content of liquid SEDDS formulation batch LP4 was found to be highest as 98.81%. So it was considered as obtimized batch for further evaluation.

Table 4: Drug content of liquid SEDDS formulation.

Sr. No.	Batch	Drug content (%)
1	LP1	98.32
2	LP2	97.56
3	LP3	98.79
4	LP4	98.81

Formulation of solid SEDDS

From the evaluation of liquid SEDDS it was observed that LP4 was optimized batch so LP1, LP2, LP3, LPV4 batches are converted to solid SEDDS to avoid stability problems. Solid SEDDS formulations were prepared by using the carrier i.e. Aerosil 200. Optimized liquid SEDDS converted into solid SEDDS by using adsorption technique. The solid carrier demonstrated to be effective to construct free flowing powder form of liquid SEDDS with high surface area. The amount of carrier required to absorb the liquid SEDDS was strongly associated with the surface area of adsorbant.

Table 6: Formulation batches of solid SEDDS.

Batch code	Liquid SEDDS (mg)	Aerosil 200 (mg)	Total wt. of tablet (mg)
LP1	350	250	600
LP2	350	250	600
LP3	350	250	600
LP4	350	250	600

*Calculation for one dose; batch size 20 tablets

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Evaluation of Solid self emulsifying drug delivery

Flow properties

Flow properties of solid SEDDS such as angle of repose, bulk density, tapped density, carr's index and hausner's ratio are deteremined and found that the prepared solid SEDDS showed "Good" flow properties as showed in Table 7.

Table 7: Flow properties of Solid SEDDS.

Batch	Bulk density (gm/ml)	Tapped density (gm/ml)	Hausner's ratio	Carr's index	Angle of repose
LP1	0.77	0.75	1.1	9.09	27.75
LP2	0.81	0.76	1.06	15.71	29.05
LP3	0.76	0.65	1.26	7.89	27.14
LP4	0.81	0.70	1.09	13.58	26.56

Effect of dilution on solid SEDDS

The formulation LP4 has found to have "Good" dilution than that of other formulations.

In-vitro dissolution test

In-vitro drug released was performed between marketed formulation and optimized batches. The in-vitro dissolution of prepared solid SEDDS was compared with marketed formulation (LOSAR®). From the result it was observed that LP4 shows more release compared to the marketed formulation these findings conclude an enhancement of permeability and, as a result the improved bioavailability of Losartan potassium.

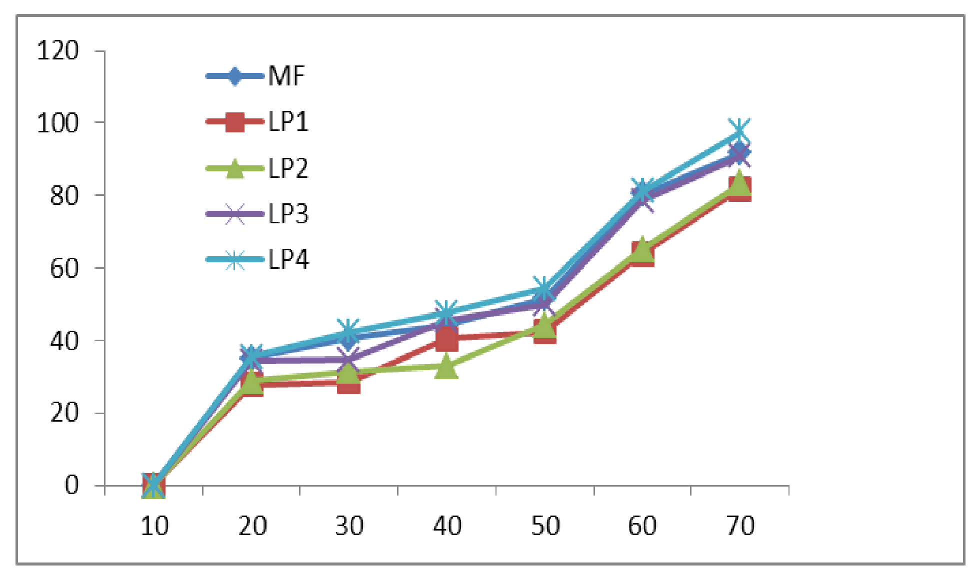

Fig. 11: In-vitro drug release.

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Drug excipient interaction by ATR-FTIR

From the observation of all FTIR spectra, it was evident that all important peak of losartan potassium and the excipients used were located in the solid SEDDS. Hence it could be concluded that there was not any chemical interaction between the drug and excipients.

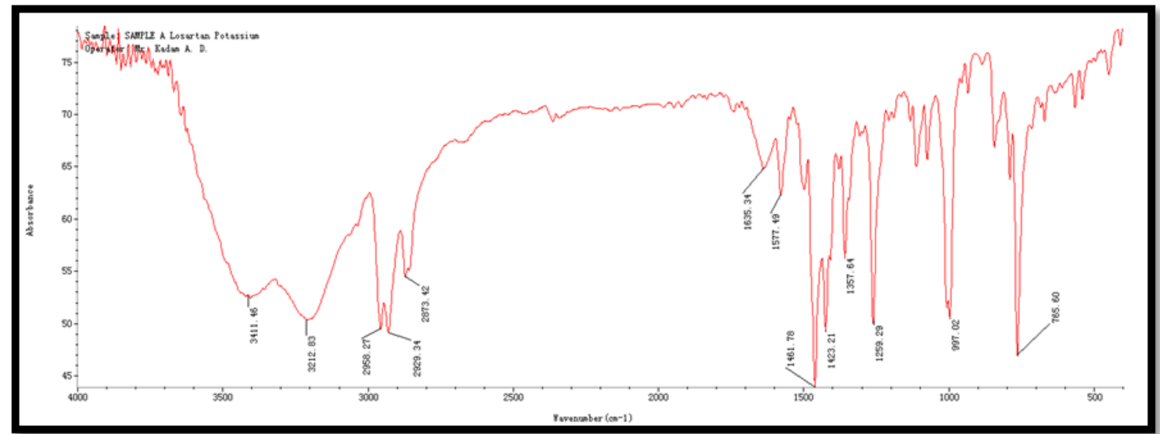

Fig. 12: Infrared spectrum of Losartan potassium.

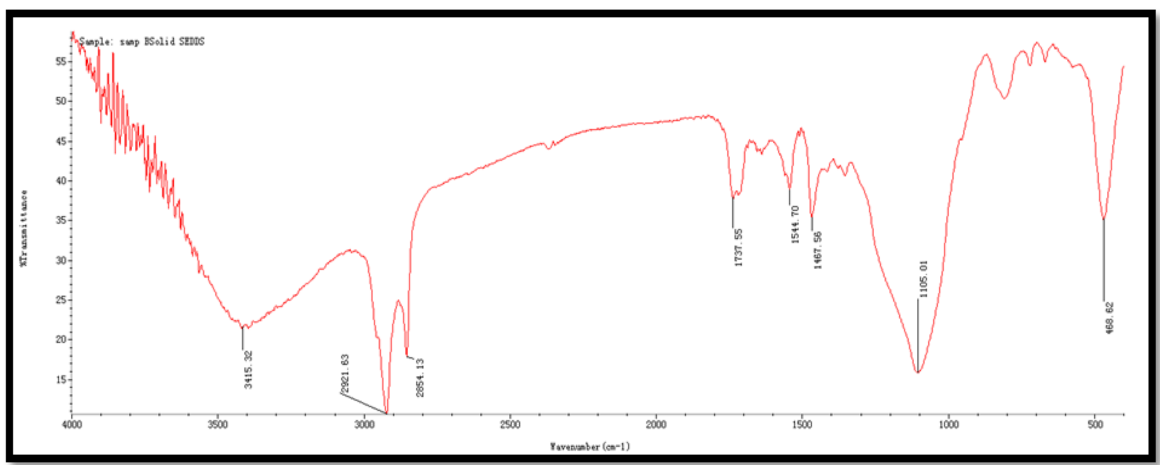

Fig 13: Infrared spectrum of solid self emulsified drug delivery system.

CONCLUSION

Liquid SEDDS of losartan potassium with oleic acid as oil phase, SDC-PC as surfactant and PEG400 as co-surfactant was successfully developed. Based on thermodynamic stability study, self emulsification time, % transmittance, zeta potential, particle size study LP4 formulation was selected. Based on above studies it was concluded that SDC-PC can be used as surfactant in SEDDS which shows good results. So LP4 formulation further converted to solid SEDDS using Aerosil 200 as a carrier and evaluated for the flow properties, effect of dilution, in-vitro drug release and FTIR studies. % drug release of solid SEDDS was almost similar to marketed formulation LOSAR®.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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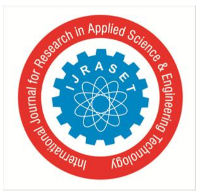

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Chatbot for Children Assistance

Ankita Dadaso Raskar[1], Akshata Santosh Dongare[2], Afrin Salim Inamdar[3], Rutuja Bharat Kamble[4], Pranali Laxman Patil[5], Dr. S V Balshetwar[6]

Abstract: Children of age 3 to 7 are unaware of language and they face trouble while learning new things so this chatbot application is designed for them to make their start towards learning easy and in interactive way. This application will help them to learn basic things required in daily life and also will entertain them. This application is helpful to enhance their skills. This is designed to provide an interactive learning medium which results in fast progress of child.

A chatbot is artificial intelligence (AI) software that can simulate a conversation (or a chat) with a user in natural language through messaging applications, websites, mobile apps or through the telephone. A chatbot is often described as one of the most advanced and promising expressions of interaction between humans and machines. Machine Learning and artificial intelligence are fast growing technologies and are used in any area to make human activities easy and fast. Chatbots are way more than simple conversational agents. They can be connected to various APIs which will for example enable them to deal with a wider range of children requests.

Multifunctional chatbot assistance built using this technology will help children in day to day activity. During 19 pandemic some issues are raised as big concern one of them is children health and growth. Parents are unable to give their proper attention to their child due to work pressure, work from home.

Chatbot assistance will help them out in daily activities and give guidelines which will beneficial to their health and growth. Chatbot will work as a their study partner.

I. INTRODUCTION

During Covid 19 Pandemic for online study of primary school students Government of Maharashtra developed a whatsapp chat assistance called "Convegenius". It was beneficial for student during weekly test as it was interactive. Students got familiar with it easily. There is no any other such application available for students below age of seven which help them in study and their daily activity. So we propose making of a voice chatbot for children of age group 3 to 7 to assist them in their activity and bind them to study with entertainment. And the main motivation we found that the majority of a chatbot users it gives a motivation for using a chatbot. It was very effective and efficient to use. Machine Learning and artificial intelligence are fast growing technologies and are used in any area to make human activities easy and fast. Multifunctional chatbot assistance built using this technology will help children in day to day activity.

Children assistant is very useful for children and it is very innovative for them. It facilitates help to do daily work of children and their studies also. This is help children to solve their different questions and also solve health issues between them .It is also helpful for their parents to overcome the care for their children. At present , children are also familier with the every technology so,our project is very helpful for them to make their intertainment medium helpful

II. LITERATURE REVIEW

Today virtual assistance are boosting technology and are used in various field. They are easy to install and access so used widely due to their flexibility. While studying Chatbot system we found that it would be beneficial for children as they love interactive sessions. So we studied different research paper on various chatbot applications to understand basic concepts and terms related to chatbot.

As per [1] Author has developed smart college chatbot using machine learning and python as a channel for information distribution. This project will investigate how advancements in Artificial Intelligence and Machine Learning technology are being used to improve many services. For human language processing they used Natural Language Processing (NLP) ("NLP: ability of computer to understand, analyze, manipulate, and potentially generate human language."). For some features they have also focused on Artificial Intelligence Markup Language (AIML) ("AIML: AIML is an XML dialect for creating natural language software agents").

As per [2] author has focused on design and development of an intelligent voice recognition chatbot. The paper presents a technology demonstrator to verify a proposed framework required to support web based bots. This online chat system follows client server approach. Voice recognition process has two parts capturing and analysis of input signal which allows the server to generate response faster.

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When comes to health generating response is more crucial and needs the high accuracy in [3] we studied the chatbot system for conversational healthcare service. In project the way they have distributed tasks and deep analyzing of data was quite impressive. They have made the module to understand user which is beneficial in response generation.

As per [4] author has focused on dialog management approaches and tools with respect to the different aspects like capability of creating natural, robust and complex dialogs, convenience for developers, scalability, reusability. On analysing these goals they had proposed the dimensions of analysis such as dialog structure, learning, error handling, dependencies, control, domain independence, and tool availability.

As per [5] author has outlined interpersonal assistants as a promising model that conversational agents may evolve. They also mentioned some elicited key functional elements for always-on services running on resource-scare devices. This helped to understand how to keep our assistant always active in proper manner.

As per [6] - this paper illustrate a web based infrastructure of architecture for conversational agents equipped with a modular knowledge base. It focuses on the enhancement of the agent interaction capabilities. From paper we study about web based chatbot and their infrastructure.

As per [7] author G. Pilato, A. Augello and S. Gaglio, in paper "A Modular Architecture for Adaptive ChatBots," has illustrated architecture for a conversational agent based on a modular knowledge representation. This paper focus on accurate response for query in effective manner to make conversation more attractive.

As per [8] automatic chatbot knowledge acquisition method from online forums is presented in this paper. It includes a classification model based on rough set and the theory of ensemble learning is combined to make decision.

As per [9] this paper presents a survey on similarities, differences, and limitations of the existing chatbots, it also presents a survey on existing chatbots and techniques applied on it. This gives the clear idea of continuous evolution of chatbot assistant. This paper helps us to understand current limitations of chatbot system. Also we got the knowledge various technology used in different types of chatbots.

This paper [10] "Review on Implementation Techniques of Chatbot," provides a critical review of chatbots and the current strategies are executively explored and talked discussed. This paper is also based on comparison of different chatbots implementation techniques. From paper we get idea for better implementation of chatbot.

Table 1. Comparison Table

Sr. No.	Paper Name	Publisher	Techniques	Merits	Demerits
1	Smart Collage Chatbot using ML and Python	H.K.K, A .K. Palakurthi , V .Putnala , Dr. Ashok Kumar K	Here artificial intelligence, machine learning, natural language processing techniques are used	1. This paper provide more user interactive as it responds to the user queries. 2. As the paper the feedback is stored in the database which can used by collage to know how efficiently chatbot is answering user queries.	This paper only show that user can ask any numbers of queries to chatbot system regarding collage.
2	An intelligent Web-Based voice chatbot	S.J.du Preez,Student Member,IEEE,M.Lall2.S.Sinha3,MIEEE ,MSAIEE	Here Artificial Intelligence,XML ,JAVA,AIML,ALICE techniques are used	This paper shows environmental facilitating transparent and high performance of the overall system	This paper shows that all modules can not running off one system.
3.	Chatbot as Conversational Healthcare Services	Mladan Jovanovic, Marcos Baez, Fabio Casati	Here shared design metaphor technique used	The paper shows that how chatbot easily interact with human/patient.	The paper only provide medication reminder not a medicines to a patients.

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4	Approaches for Dialog Management in Conversational Agents	J. Harms, P. Kucherbaev, A. Bozzon and G. Houben,	Hera automatic speech recognition, artificial intelligence, machine learning are techniques used	1. This paper provides an overview of the state-of-the-art of commercial as well as research tools. 2. This paper shows opportunities for future research directions.	This paper shows memory network research is not mature enough yet to explore domain independence.
5	Towards Interpersonal Assistant: Next Generation Conversational agent	Inseok Hwang, Chulhong Min Youngki Lee , Dangsun Yim, Chungkuk yoo John Kim.	Here shared design metaphor techniques used and Artificial Intelligence are use	1. It trains the parents and develops the language of child. 2. Frequently changing speech characteristics as they grow, and insufficient speech carpus specialized in children.	1. This paper provides prominence of micro structural properties is not limited to a particular example of interpersonal assistants.
6	A Modular Framework for Versatile Conversational Agent Building	A. Augello, M. Scriminaci, S. Gaglio and G. Pilato	Here conversational agents, modular KB, ontology reasoning, semantic spaces techniques are used	This paper provides the information about architecture and how to exploit different modules suited for specific dialogue requirements.	The test script have test data embedded in them, which will become problem when updating the code
7	A Modular Architecture for Adaptive Chatbots	G. Pilato, A. Augello and S. Gaglio	Here artificial intelligence, Corpus Callosum techniques are used	This paper provides intelligent conversational agents with a dynamic and Hexible behaviour.	Speech acts are detected through a simple rule-based speech act classifier whose description goes beyond the scope of this paper.
8	Automatic Chatbot Knowledge Acquisition from Online Forum via Rough Set and Ensemble Learning	Y. Wu, G. Wang, W. Li and Z. Li	Here Forum, Multiple rough set techniques are used	This paper shows high recognition efficiency to related replies and the combination of ensemble learning improve the result.	Approaches are not capable of extracting knowledge for specific domain.
9	A Survey on Chatbot Implementation in Customer Service Industry through Deep Neural Networks	M. Nuruzzaman and O. K. Hussain	Here Neural Network, Peep learning, Natural language processing, Dialogue system techniques are used	This paper provides sequential attention mechanism in deep recurrent neural networks.	Existing chatbot do not have an interactive user interface and maintain poor documentation.
10	Review on Implementation Techniques of Chatbot	S. Nithuna and C. A. Laseena	Hare AIML, ALICE,DNN, machine learning, natural language processing techniques are used	In this paper critical review of chatbot and current strategies are explored.	Hard to understand the framework effectively with no rules to visitors.

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III. CONCLUSION

Chatbot assistant or virtual assistant are fast growing technologies having large impact on different industries. We have variety of virtual assistant available in market which helps to reduce the human efforts as they are self-learners from conversation and automated. There is no need to invest our time in their tasks as once it started learning it start becoming better and better.

While building a chatbot first we have to create proper sequence of conversation as it is the heart of system. With proper flow of dialog system becomes more attractive. Choosing correct technology is crucial part of building a chatbot. We have make our system with respect to current technology used in industry and update its functionality to make it compatible with respect to time. Also the starting and ending of conversation is important to make impression on user. We have to focus on requirement of end user and try to fulfill them. Adding feelings and improve relevant answers is must to make chatbot appear as real person.

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Smartphone User Behaviour Predication Using AI

Mayur Ramesh Chavan[1], Akshay Avadhut Kulkarni[2], Mohammad Kaif Shakil Mulla[3], Ashish Hanmant Mahadik[4], Omkar Jagnnath Waghmare[5], Dr. S V Balshetwar[6]
[1, 2]Dr. Daulatrao Aher College of Engineering Karad

Abstract: There are lots of Smartphone users data floating around which is not efficiently used for improving user experience and which can be used to provide a better user experience or enhance the current one. Enhancing user experience is a challenge as there are multiple competitors. A persons time spend on their Smartphone is an vast amount of time and everyone is trying to reduce this time by implementing efficient ways of android user experience. Starting with our work, which will focus on this user floating data? There are important segments of this user data one of them is facial data because humans are used to taking in non verbal cues from facial emotions. And also we are focusing on factors like user surrounding sound, user's locations and patterns of application used history.

I. INTRODUCTION

As in the 21st century Electronics devices are conquering the whole world and use of Smartphone is increasing day by day in which most of the time spent on smart phone is less productive, whereas it leads to wastage of time. So while studying an average Smartphone user we found that the Smartphone can cause two different problem, where first one shows a negative and second one shows positive impact of Smartphone . Coming to the first problem shows us the deficiency.

So now we will discuss the first problem and further continue to explain second problem. There are lots of Smartphone user's data floating around which is not efficiently use for improving user experience and which can be used to provide a better user experience or enhance the current one. Enhancing user experience is a challenge as there are multiple competitors. A persons time spend on their Smartphone is an vast amount of time and everyone is trying to reduce this time by implementing efficient ways of android user experience. Overusing pattern of Smartphone involves a tendency to check notifications all the time. Such behavior pattern can induce "reassurance seeking" pathway which broadly includes symptoms such as loneliness, low self-esteem, depression, and anxiety. Excessive use of Smartphone may also affect sleep patterns by reducing rapid eye movement sleep, slow-wave sleep and consequently causing sleep.

II. LITERATURE REVIEW

Coming to the first problem discussed above that is the overusing pattern of Smartphone involves a tendency to check notifications all the time. Such behaviour pattern can induce many health problems. After studying these problems we found that there can be a single solution, which will be focusing on prediction of an average Smartphone user behaviour. As this can be a challenging task because there are varieties of peoples from different age group, different background, etc.

So we decided to focus on a specific group of people which are "Students". As student are the most suffering from Smartphone addiction as they are in there early years of education life. Student may feel overwhelmed, curious, lonely, bored, stressed, depressed or even anxious about something. Once they enter in virtual world of Smartphone applications, they might feel to forget worries and feel better. But this can further lead to addiction, as student can make Smartphone as comfort zone and spend more and more time on Smartphone. Smartphone is a device that brings the world closer to us but excessive use of it can disturb real world connections.

As we have studied problems related to Smartphone user behaviour found some solutions and these solutions are highlighted are in our research. In [1] Ankita Kanhangadand and et.al.in there paper had taken the data from the sensor like orientation sensor, accelerometer sensor and gyroscope sensor which are built-in sensors. And from these dataset taken from the various sensors they are going to only predict the human behaviour. The analysis of human behaviour has been proven to be effective in various applications including biometricbased user authentication, smart spaces, human-machine interactions, physical activity recognition and surveillance. The advantage of this thing is human behaviour is captured unobtrusively without requiring a conscious effort on the part of user. This study will help us to explore techniques that can be used to predict human behaviour.

As per [2] Subrata Tikadar and Samit Bhattacharya are it is very important to know user behaviour to design and built effective interaction system, tools or application.

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The behavioural study not only helps to assure the success of any design or product but also helps other researchers from various related areas. They have systematically collected and analyzed the behavioural data for Smartphone usage by 1711 students of 188 academic institutions throughout India.

They have also observed students behaviour on Smartphone usages both inside and outside the classroom. They have also found dissimilarities and are also expecting that the findings of the study will help many researchers from various fields including HCI, Mobile HCI, Behavioural Science, Psychology, and Education. This study will help us to know importance of prediction of human behaviour and explore its depth knowledge.

As per [3] authors have here presented the article, which present the frameworks for mobile audience measurements, for collecting data at the point of convergence - devices.

This paper compares the presented framework to alternative methods of mobile user research, and identifies the unique advantages of on-device measurements along with the key weaknesses. This study shows us that there are various techniques of mobile audience measurement from various aspects through a Smartphone.

In[4] Ruxia hong has focused on the research of mobile user behaviour based on big data and it has become one of the hotspot in the field of internet, Every internet user leave their footprints.

It is based on the analysis of the characteristics of mobile user's big data and hadoop system, an analysis model of mobile user's behaviour pattern based on big data is constructed, it includes data acquisition module, data pre-processing module, user behaviour analysis module, application of mobile user behaviour model and data visualization module, and the function of each module is explained in detail. This study shows us that big data can play a measure role to analyse data sets and also that internet can be a vast plays to explore Smartphone user data.

As per [5] author has given the model of machine learning for activity recognition and authentication of Smartphone user.

As per Authors technological advancements have made Smartphone's to provide wide range of applications that enable users to perform many of their tasks easily and conveniently, anytime and anywhere. As per their study many users are tend to store their private data in their smart phones.

Since conventional methods for security of Smartphone's, such as passwords, personal identification numbers, and pattern locks are prone to many attacks, this research paper proposes a novel method for authenticating Smartphone users based on performing seven different daily physical activity as behavioural biometrics, using Smartphone embedded sensor data. This authentication scheme builds a machine learning model which recognizes users by performing those daily activities. This study shows us that Smartphone sensors can be combined and used for user identification to increase security measures.

As per [6] Balaji Balasubramanian and Pranshu Diwan et. al. signify in their paper that human beings relay a lot on non-verbal communication and facial emotion in large. In this Paper they cover the dataset and algorithm that are used for Facial Emotion Recognation(FER). And the algorithms range from simple Support Vector Machines (SVM) to complex Convolutional Neural Network (CNN). This paper shows us importance of facial emotion and how it can play a measure role in our work since they have also represented various techniques for recognising it.

As per [7] Richard Han, Mahnaz Roshanaei and Shivakant Mishra all authors present in this paper have formed a group of 20 peoples for a research that put in the picture how every individual from the group reacts in different scenario that relates with respect to their Smartphone. They experimented most of the possible scenarios and prepared data sets to be tested with machine learning algorithms for the better accuracy to knowing their behaviour.

As per [8] Natasha Jaques, Sara Taylor, Asaph Azaria and et.al. focused in this paper that an average teenagers happiness depends on some of their close things that also includes there Smartphone's and other things like sleep. These things can affect measure things in their life and can also result in their personal losses and they also found 70% accurate module that shows this behaviour. This study can help us with our research to know about this factors and there consequences.

As per [9] mobile phones are equipped multiple sensors from which there are new opportunities to analysis user's daily behaviours and also how truly intelligent personal devices are.

They have proposed a MAST (movement, action & Situation over Time) model to explore along this direction and identify key technology required. And also they have found an idea of reducing power consumption for mobile phones with the help of phone-cloud collaboration model. This study shows us a new model known as MAST and it can be very valuable in our survey.

As per [10] Smartphone have lots of resources that can be utilized to enhance user behaviour analysis. Study shows that how Psychological Science can be used to study user behaviour, it gives an idea about various opportunities for analysing Smartphone and it can be helpful in our work.

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Below Table1.1 shows comparison of different techniques used to analyse Smartphone user behaviour

Table 1. Comparison Table

Sr. No.	Paper Name	Publisher	Techniques	Merits	Demerits
1	Smartphone usage contexts and sensible patterns as predictors of future sedentary behaviours	Qian He, Emmanuel O. Agu	Here logistic Regression technique is used	1.With the help of logistic Regression they are able to classify user context variable such as location, time and app usage. 2.The paper also shows that users are very sedentary or not, means in other words how much user is spending time as seated.	The paper is only limited to students and student age group only.
2	Smartphone app usage as a predictor of perceived stress levels as workspace.	Raihana Ferdous, Venet osmani, Oscar Mayora	Here predicting a stress level of user based on smartphone app usage technique is used	1. By understanding the patterns of app usage and investigating relationship of these pattern the perceived stress level within the workspace context. 2. The result they have achieved is average of accuracy of 75% and precision of 85.7% can be used as an indicator of over all stress level in work environment.	This paper only show smartphone user stress levels and it is not enough to predict it next move.

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3	Machine Learning models for activity recognition and authentication of smartphone users.	S. Sareh Ahmadi, Sherif Rashad, Heba Elgazzer	Here normal technique for security of smartphone and conventional method for same is used.	1. This paper tend to provide a better way to increase security of users data. 2.With the help of seven different physical activities like behaviour biometrics, using smartphone embedded sensor data security is provided in the sense of passwords, personal identification numbers, and pattern locks etc.	The proposed way of security requires 7 daily activates and it leads to more time consumption.
4	Artificial Intelligence and Mobile Phone Sensing based User Activity Recognition	Chia-Liang Chen, Fu-Ming Huang, Yu-Hsin Liu, DaiEn Wu	Here four supervised machine learning technique is used and various classification models are made	1. With the help of logistics regression, and support vector machine automatic activity classification model is created. 2. They have evaluated the prediction performance and the results of these experiments shows that under specific acceptance of accuracy and minimum model training time, the decision tree algorithm creates the best model.	This paper only show the best algorithm for User Activity Recognition but not tend to give an patter or prediction.
5	Identifying smartphone users based on how they interact with their phones	Mohammed A. Alqarni, Sajjad Hussain Chauhdary, Maryam Naseer Malik	Here also they had used the technique of machine learning various algorithms, Gesture recognition and behavioural	1. The maturity in sensor chips and machine learning algorithms provides a better solution for authentication problems based on behavioural biometrics, which aims to identify the behavioural traits that a user possesses,	This paper tends to use more physical activities than use of smartphone itself.

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			biometric is also used.	such as hand movements and waving patterns etc. 2. Their research study aims to provide a solution for passive and continuous authentication of smartphone users by analysing their activity patterns when interacting with their phones.	
6	Is Smartphone usage is truly smart? A Qualitative investigation of IT addictive behaviour	Liette Lapointe, Camille Boudreau-Pinnsonneault, issac vaghefi	Here they have used a grounded theory to report the result of addictive smartphone usage.	1. As per the investigation i.e. the 11 depth interviews and answers to 183 exploratory questions they have revealed out of four smartphone user profiles two of these are exhibiting addictive behaviour.	The paper show the study that show if a person is addicted to smartphone or not but it does not specifies it types or any further details.
7	A Smartphone user activity prediction framework utilizing partial repetitive and landmark behaviours	Peng Dai, Shen Shying ho	Here they have made their own technique/algorithm to find a repetitive behaviour of smartphone user.	1. With the help of Activity Prediction Framework they are giving prediction of next day behaviour of same user based on weighted sum of most similar behaviour vectors related to landmark behavour of next day behaviour. 2. With the help of arbitrary call activity, voice call activity, short message activity, media consumption and app usage datatypes, extensive experiment are carried out using nokia mobile data challenge (MDC) dataset to demonstrate the	The study conducted through this paper is based on old datasets that will not be useful that much.
				feasibility of their proposed approach.	

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8	Usage Prediction and Effectiveness Verification of App Restriction Function for Smartphone Addiction	Katsuki Yasudomi, Toshitaka Hamamura, Masaru Honjo, Akio Yoneyama, Masato Uchida	This is the study paper which focuses on app restriction function.	1. They are focusing on app restriction function, which is one of the key features of digital medicines for smartphone addiction, and analyze the usage of the function and verify its effectiveness. 2. Their results showed significant differences in both psychological and behavioral aspects between those who used the app restriction function and those who did not. Specifically, they found that the app restriction function is more likely to be used by those who were more aware of their smartphone addiction.	The paper show study that only focuses on one method for smartphone addiction.
9	Human Behaviour Impact to Use of Smartphones with the Python Implementatio n Using Naive Bayesian	Iftakhar Mohammad Talha, Imrus Salehin, Susanta Chandra Debnath, Mohd. Saifuzzaman, Nazmun	Here they have used Naïve Bayes theorem to learn impact of hum an behaviour.	1. They have found out the major problem of the human behaviour's negative side and its different sources like mental imbalance, stress, depression, loneliness, etc. 2. With the help of naïve bayes theorem and classifier, support vector machine, special data set of human behavior, and probability are used to calculate accuracy.	The paper only focuses on the accuracy of the system and does not tends to give an specific output to human behaviour with respect to smartphone.

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10	Human Behaviour Analysis through Smartphones	Kostas Konsolakis, Hermie Hermens, Claudia Villalonga, Miriam VollenbroekHutten	This is also actually a survey paper which is addressing the human behaviour analysis.	1. This paper surveys the state-of-the-art in human behaviour analysis based on smartphones. 2. They have categorize prior works into four main sensing modalities related to physical, cognitive, emotional and social behaviour which will help the smartphone user to improve themselves.	The tends to only understand human behaviour and doesn't tends to use this analysis for Furter prediction proposed or else.

III. CONCLUSION

A. The task of manually performing every search, every navigations involves a quit amount of time and can be also frustrating task.

B. So this study shows us that a simple suggestions that follows the predictions done by the AI can make a difference in users smartphone experience.

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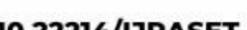

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Blockchain based record date management system using artificial intelligence

Dr. Girija Gireesh Chiddarwar
Marathwada Mitra Mandal's college of Engineering,
Pune, Maharashtra, India.
Email-id - girijachiddarwar@gmail.com

Dr. Sarita V Balshetwar
YSPM'S, YTC, Faculty of engineering,
Satara, Maharashtra, India.
Email: balshetwar.satara@gmail.com

Dr. Bharati P. Vasgi
Marathwada Mitra Mandal's college of Engineering,
Pune, Maharashtra, India
Email-id - bharativasgi@gmail.com

ABSTRACT

Medical care related innovation has been developing dramatically, from electronic medical care records (EHRs) and individual wellbeing trackers to populace wellbeing the executive's instruments. Presently, information from these sources is generally chronicled, dissected independently or utilizing just careless coordination with different information sources. In this review, we propose a coupled artificial intelligence Blockchain EHR the executive's framework. The objective is to give a stage that influences blockchain and man-made brainpower (man-made intelligence) for (I) secure EHR the board, (ii) effective information coordination, and (iii) solid PC helped analyze. An objective situated demonstrating approach with the Obliged Objective Model (CGM) is utilized to evoke the framework prerequisites. Survey results for a contextual investigation in Abu Dhabi, UAE served for model approval and refinement for expanding the quantity of framework clients. Electronic Wellbeing Records (EHRs) are electronically-put away wellbeing data in a computerized design. EHRs are ordinarily divided between medical care partners and face power disappointment, information abuse, absence of protection, security, and review trail. Then again, blockchain is the progressive development of the 20th century that offers a disseminated and decentralized setting to convey among hubs in a rundown of organizations without a focal power. It can address the limits of EHRs the board and give a more secure, got, and decentralized climate for trading EHRs information. Three classifications of blockchain-based potential arrangements have been proposed by scientists to deal with EHRs: calculated, model, and carried out. This study zeroed in on an Efficient Writing Survey (SLR) to find and break down articles submitted either reasonable or executed to oversee EHRs utilizing blockchain. The review inspected 99 papers that were gathered from different distribution classifications.

Keywords — blockchain, healthcare record management, artificial intelligence, goal-oriented requirements engineering, AI Artificial Intelligence, Machine Learning, Internet of Medical Things, Machine to Machine, Attribute-based Encryption.

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INTRODUCTION

THE fundamental plan of electronic medical care records (EHRs) neglected to expect the requirement for the administration of multi-institutional, lifetime medical care records. The ordinary difference in medical services suppliers renders patient information scantily followed across various sources. Thus, patients frequently need simple admittance to their noteworthy information, while suppliers hold essential possession. Characterizing medical care information trade instruments and pathways is especially difficult, however guarantees profoundly certain inputs for medical services framework activities and clinical exploration [1]. Protection concerns are universal among medical care establishments and are much of the time the primary driver for their shut information strategies [2], alongside the danger of information divulgence to their upper hand.

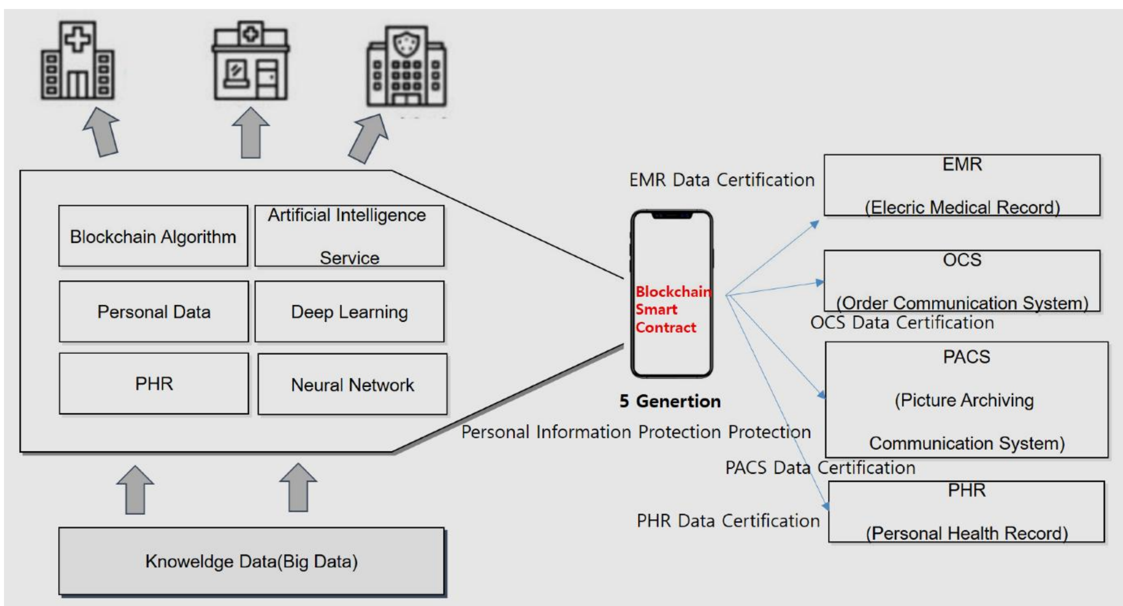

Fig.1:Blockchain based record date management system using artificial intelligence Process.

Lots of medical care information would be exceptionally advantageous for medical services suppliers whenever dissected. These information can help us in battling the infection through clinical help, early notice, and proposal [8]. Notwithstanding, it has turned into a major test for scientists to store and dissect wellbeing information in light of the fact that most are deficient and blemished. In this manner confirmation and approval of such information are pivotal for revealing, and suggestion [6]. Blockchain innovation has incredible possibilities to handle the pandemic emergency. It can assist with building a decentralized information global positioning framework that can be recovered when important. Furthermore, this enormous medical care information, particularly EHRs, is defenceless against protection and security breaks. Beginning from the Coronavirus episode, medical care suppliers and scholastic associations confronted a few complex cyberattacks [1]. The Worldwide Lawbreaker Police Association (INTERPOL) distributed a report about digital assaults connected with Coronavirus in April 2020.2 Medical services ventures have been seriously impacted close by others by these assaults. On 6 May 2020, INTERPOL delivered a mindfulness crusade where different digital assaults during pandemic were listed.3 accordingly, it is significant to do whatever it may take to handle these dangers.

Medical care record information is the essential wellspring of data and the establishment for clinical examination.

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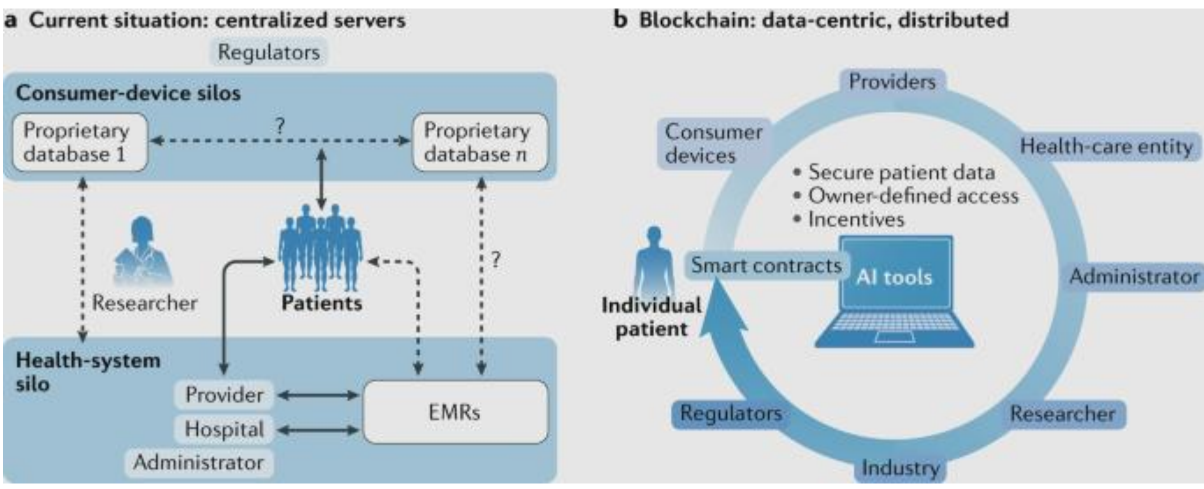

Fig.2: Blockchain based record date management system using artificial intelligence Structure.

Ebla et al. [7] accentuate that clinical and medical care analysts depend on their capacity to get to and examine multi-source information to evaluate potential collective wellbeing gambles, propose case-subordinate therapies and designated medication. While sensible measures of information are made accessible for research from clinical preliminaries, censuses and college subsidiary clinics, a striking expanding interest in huge scope information trade exists across different key partners [8]-[1]. The point of this study is to frame the objectives and necessities for a coupled man-made intelligence blockchain EHR the board structure. The work depends on survey criticism from three emergency clinic bodies across the UAE and applies the Obliged Prod Demonstrating (CGM) approach with its related CGM-Device [1] .The general progress of the framework relies basically upon the client movement - the quantity of exchanges. Notwithstanding, the proposed framework is intrinsically made out of numerous goals, capabilities, and partners.

Model fundamentally not the same as the authorization less mining in the Medrec system. They decided to create a shut, access-controlled blockchain EHR framework from a clinical perspective. This thusly elaborate the reception of distributed storage and access key exchanges for encryptions, though Medrec put away tolerant information locally at each hub. In both the consent less and permissioned models, the benefit of adding simulated intelligence to the structure of a blockchain EHR the executives framework, has not been completely understood, even in the latest writing.

FRAMEWORKS

Blockchain innovation is a relationship of two advancements, cryptography, and P2P. A blockchain is a progression of timestamped blocks associated through a cryptographic hash. Regularly each block contains exchange records confirmed by the friends, called excavators. The chain is expanded consistently, and each new block is added as far as possible. Be that as it may, each new block contains a reference, essentially a cryptographic hash (e.g., SHA-256), of the past block's header. The making of each block guarantees secrecy, straightforwardness, and unchanging nature [5]. The entire situation of blockchain is held in a P2P organization. The essential construction of a blockchain. Each block with the exception of the beginning block (first block of the organization) has the hash worth of information from the past hash. Moreover, each block has a trouble esteem called Nonce, a Timestamp, and different qualities (e.g., the rundown of exchanges).

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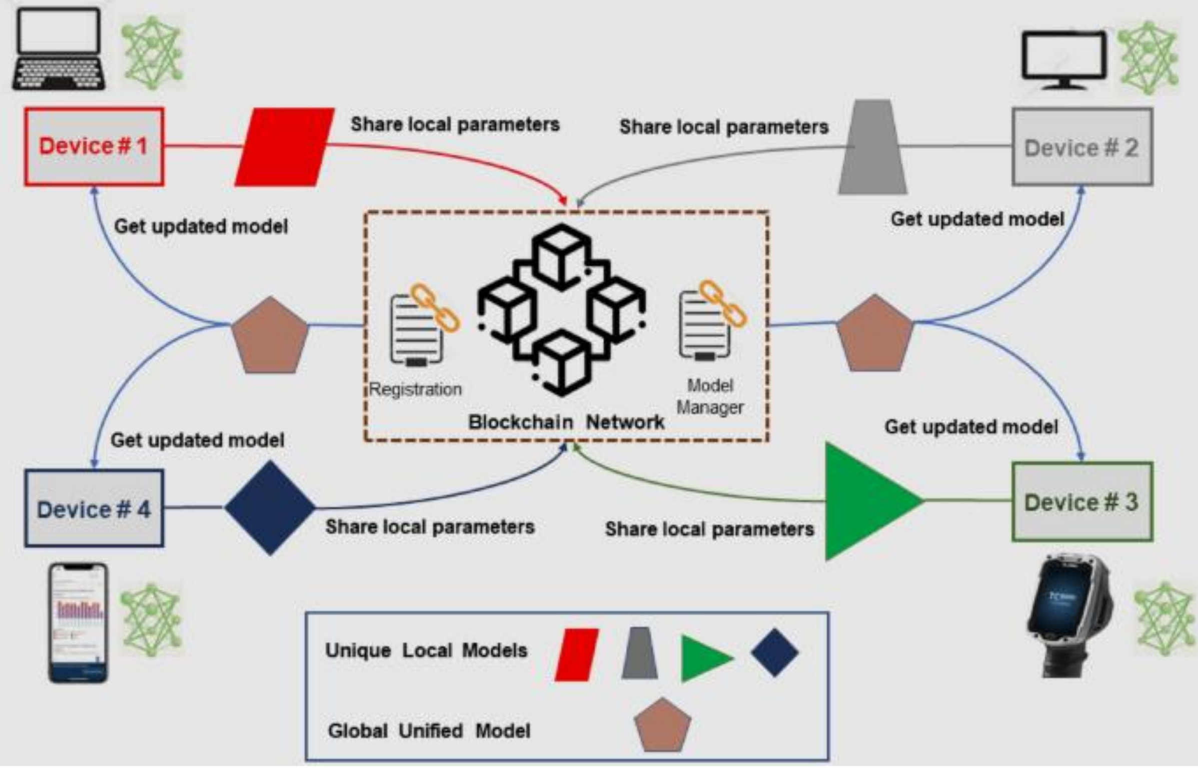

Fig.3: Blockchain based record date management system using artificial intelligence Flow.

Displaying and Information Examination the CGM was arrangement as per the stepwise methodology nitty gritty in [1] and [7]. The initial step was characterizing the objectives, refinements, and area suppositions, backed by understanding the CGM. The third step was contributing inclinations and checking great formedness. Following the finishing of stages 1 and 2, the instrument is pursued contributing inclinations to actually take a look at all kinds of situations. The "Actually look at Well-Formedness" capability, implanted in the CGM apparatus, is applied to evaluate the quality and legitimacy of the applied model, making sure that all components and their relations are appropriately connected. The mistakes to check are named "Void Chart", "Invalid Objective Hub", "Refinement Legitimacy Check", and "Undeclared Variable" [7]. The model produces situations subsequent to checking for consistency. Given the impediment of information and partner crowd, the last step of choosing the most sensible acknowledgment, as indicated by partner inclinations [7], was not performed here. The survey [8] filled in as the fundamental reference for evaluating and approving the proposed model in distinguishing the basic partners and their assumptions from using the proposed EHR the board framework.

PRIVATE BLOCKCHAIN

Confidential Blockchain has a few similitudes with a public one regarding activity and calculations. Nonetheless, it contrasts in reason. In basic terms, a private blockchain is a prohibitive or permissioned blockchain. It is worked in light of some entrance control rules in a shut organization, which is circulated at this point unified. This sort of blockchain is normally utilized inside an association or organization where at least one hubs control which hub can perform exchanges, go about as diggers or perform savvy contracts. The security, openness, consents, and approval are constrained by a TTP association.

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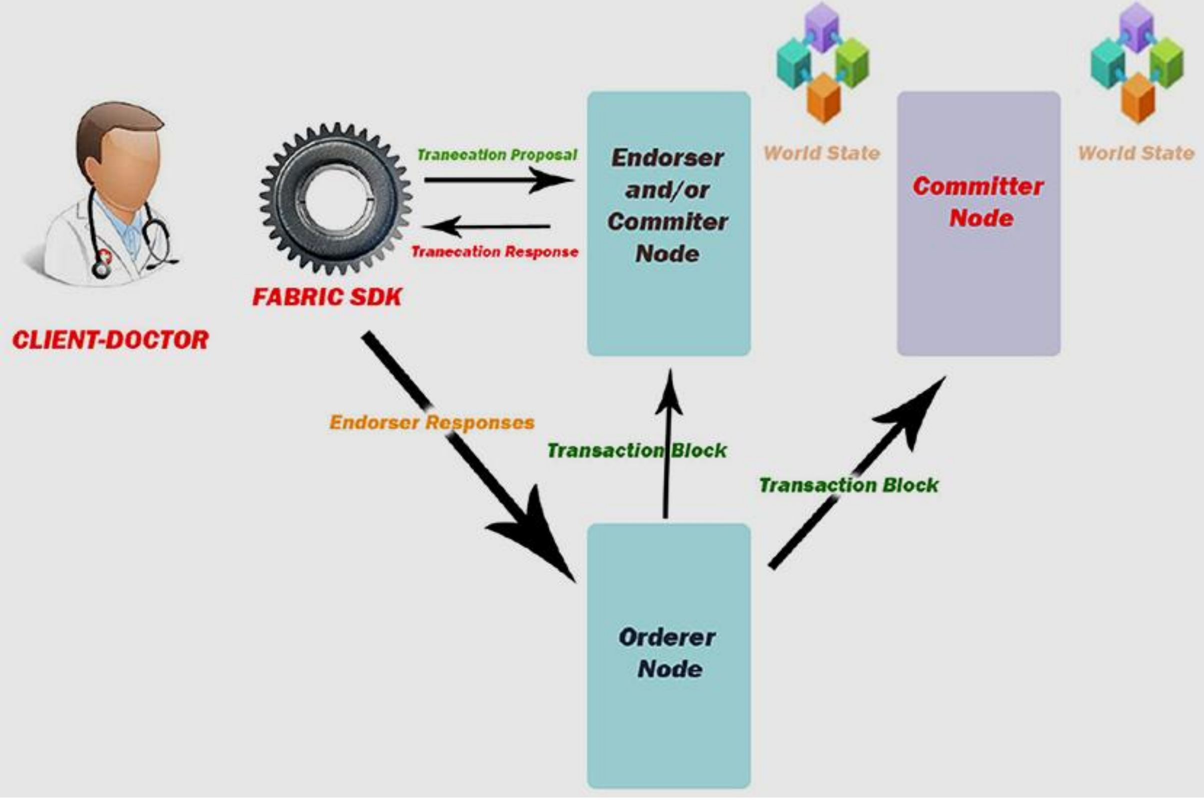

Fig.4: Blockchain based record date management system using artificial intelligence.

This kind of blockchain is utilized regularly for store network the board, electronic democratic, advanced resource the executives, and information protection. Hyperledger Texture [9] and Wave [2] are brilliant instances of private blockchains. It's not possible for anyone to join a private blockchain network without a greeting from approved faculty. Moreover, it consumes less power than the public blockchain, and it is quicker in adding blocks to the chain. Subsequently, a private blockchain is recommended to oversee EHRs.

RESULTS AND DISCUSSION

The aftereffects of the CGM instrument demonstrating show the obligatory parts and their interlinkages to inspire the achievement pathways of the proposed structure. The essential practical necessity was Augmenting Utilization of HR Blockchain Framework. Five significant level middle objectives are characterized as: Implementing Blockchain, Giving Devices, Expanding Security, Diminishing Exchange Expenses, and Expanding Interoperability, alongside the accompanying non-practical (pleasant to-have) necessities: New Positions, Engaging Youth, Worked on General Wellbeing, Getting Close Family HRs, and Decreased Exchange Time. The general model is portrayed, which represents the principal prerequisites and their interconnections for expanding the utilization of the blockchain EHR framework. Meanings of Prerequisites, Moderate objectives, Undertakings, Area Suspicions, and Refinements can be found in [1] and [7].

Transitional objectives, undertakings, as well as space suppositions that, together, uncover the intricacy related with sending a fruitful simulated intelligence blockchain EHR the board framework. Tending to Q2, the obligatory necessity of Boosting Utilization of HR Blockchain Framework was seen diversely by the poll responders. Patients were more worried about their ownership of full control of their medical care records, while others focused on the coordination of their own information (for example from wellness devices and versatile applications) with their medical services records.

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Situation Age the created model and its acknowledge were evaluated in view of the gathered reactions from the survey. Tending to Q1, the key partners got from the survey input and, subsequently, from the CGM graph of Figure 1 can be distinguished as follows: The UAE Government, including the regulation bodies and medical services guideline organizations (for example HAAD for the Emirate of Abu Dhabi), Emergency clinic Bodies, Medical services specialists, IT specialist co-ops (organizations and individual specialists), blockchain specialists, insurance agency, clinical focuses (Facilities), drug stores, and the overall population, explicitly patients. The model exhibits the centrality of participation between the referenced players for the achievement the proposed framework. The partner rundown will normally grow as the middle of the road objectives are refined past the extent of the current review, which fills in as a starter structure.

Medical services professionals distinguished more information sharing for clinical exploration just like an area of exorbitant interest. Clinic IT and information base (DB) supervisory groups distinguished information interoperability, precision and exchange time as their key worries. While not unequivocally recorder in the study, the major drivers for taking on blockchain in the UAE is the public authority's consistent endeavors toward mechanical progression and advancement [9]. A sum of thirteen anonymized review reactions were recorded. Regardless of the low return rate, the reactions served the model turn of events and investigations. Concerning looked in acquiring patients' EHRs, reactions included: missing data, conflicting configuration, and long exchange times. None announced sharing patient information for research purposes, albeit all reactions demonstrated their dynamic support in clinical exploration. There was consistent assent that patients ought to have sole command over their medical services records, with the state of a predictable, secure and organized climate gave.

ALGORITHMS FOR BLOCKCHAIN

The agreement calculation is a dynamic cycle in a gathering of hubs in the blockchain which should be trailed by the other hubs. To comprehend this exhaustively, let us think about the accompanying model. Assume there are 20 individuals in a conference to settle on an impending task. Everybody can propose their own perspective with respect to the undertaking, however the prospect that benefits a great many people will get a higher inclination. Essentially, in the event that we think about a digital money, e.g., Bitcoin, the excavators need to settle numerical riddles to meet POW agreement and get a few compensations as Bitcoin. In many blockchains, agreement calculations are the vote of larger part members.

CONCLUSION

The current review gives a primer structure to evoke the different intricacies and interdependencies in the execution of an artificial intelligence blockchain framework for EHR the board, explicitly in the UAE setting. CGM is demonstrated to be an important device in determining, planning, explaining, and modifying complex framework necessities. Five undeniable level middle objectives are characterized as: Upholding Blockchain, Giving Devices, Expanding Security, Lessening Exchange Expenses, and Expanding Interoperability, to fulfill the obligatory prerequisite of an effective EHR the board framework, alongside a sum of 22 undertakings. Different non-useful necessities were likewise caught: New Positions, Engaging Youth, Worked on General Wellbeing, Getting Close Family HRs, Diminished Exchange Time,

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while a few others might be become clear with additional refinement. Utilizing the underlying outcomes from the system proposed in this review, the future work on growing the examination by refining the objectives and errands with the distinguished partners is suggested. An administration embraced study is recommended to guarantee more extensive cooperation of medical services foundation for additional definitive and delegate discoveries. In the long run, a rundown of potential medical services organizations can be recognized to execute and test a functioning model of the proposed framework, as in [1] and [5]. We will likewise additionally broaden the displaying past the objective examination to envelop full necessities detail [3], and influence our past specialized work in the blockchain region

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SUITABILITY OF KINETIC ENERGY FROM FOOTSTEPS FOR VADJAIDEVI TEMPLE AT PATKHAL, TALUKA, DISTRICT SATARA

Prof. Shah. Ajinkya S[*1], Mr. Arjun Avinash S[*2], Mr. Khade Sagar S[*3],

Mr. Hakim Mohammadsabir N[*4], Ms. Desai Sayali S[*5], Ms. Mane Neha S[*6]

[*1]Guide, Civil Engineering Department, Yashoda Technical Campus, Satara, Maharashtra, India.

[*2,3,4,5,6]Students, Civil Engineering, Yashoda Technical Campus, Satara, Maharashtra, India.

ABSTRACT

For many years man has been in need of energy and he has been using it to a great extent for prosperity and sustenance. This use was responsible for lot of energy wastage. The usage energy for foot strength with movement of every human is very important for high density countries such as India, where crowded around runs on the clock. Kinetic energy using harvesting is a sustainable method for generating electricity without harm to natural resources. This study focus on harvesting of walking energy and compare with different technology used for converting walking energy into electrical energy. For this project we have chosen temple location as crowded place. The name of temple is Shri Vadjai Devi Temple at Patkhal.

Keywords: Energy Harvesting, Piezoelectric Sensors, Kinetic Footsteps, Vadjai Devi Temple, Sheet Metal. Kinetic Energy.

I. INTRODUCTION

In past few decades it has been noticed that the one way of generating energy must change due to its harmful effects on the global climate. Energy harvesting is one of the emerging methods. It is the method in which kinetic energy is converted into electrical energy. This kinetic energy is generated from human footsteps falling on a mechanical tiles. These tiles have mechanism in which there are piezoelectric sensors. These piezo sensors captures kinetic energy from human footsteps which is wasted energy. Then it is converted into electrical energy and stored for later use. Without wasting natural resources, energy harvesting is one of the promising techniques for facing the global energy problems. Harvesting energy is a developed methd from a small power generation system which refers to milli-watts. This method is for replacement or augmentation of the batteries. The kinetic energy harvesting that is the purpose of study, converts movement, mainly in form of vibrations, into electrical energy.

Kinetic energy: kinetic energy is a form of energy that an object or a particle has by reason of its motion. For example – a person walking. Kinetic energy having different types such as Thermal Energy, Sound Energy, Mechanical Energy, Electrical Energy.

Kinetic footstep: kinetic footstep **(Fig.1**) is an eternal energy source for the generation of electrical energy without usage of any natural resources.

Fig 1: Kinetic footstep

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The purpose of kinetic footstep system is to generate electrical energy by using some mechanical properties of materials and the walking of human beings.

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Location: We have selected shri vadjaidevi temple **(Fig.2)** in patkhal village for project survey. This temple was built about 90 years ago.

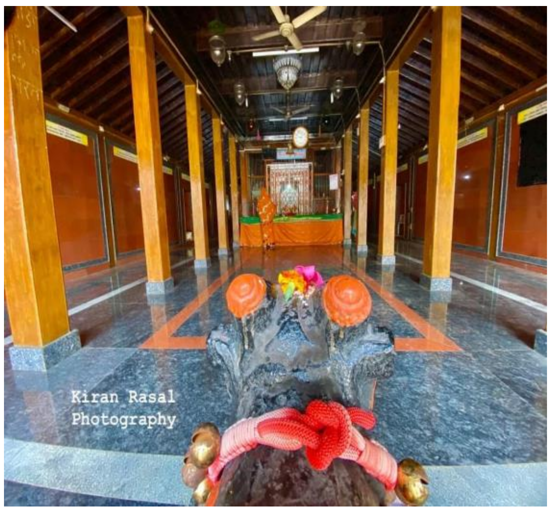

Fig 2: Shri Vadjaidevi Temple

II. OBJECTIVE

- To select the suitable site
- To create piezoelectric tiles
- To generate renewable, electrical energy by using kinetic footstep

III. MATERIALS

1. **SHEET METAL:** sheet metal is metal formed by an industrial process into thin, flat pieces. Having gauge 0.16 and size 1.15*1.5 feet with black in color.

Fig 3: Sheet Metal

2. **PEIZO SENSORS:** Piezoelectricity is the charge created across certain materials when a mechanical stress is applied. Piezoelectric pressure sensors exploit this effect by measuring the voltage.

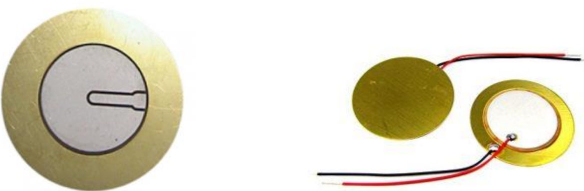

Fig 4: Piezoelectric Sensors

3. **BATTERY:** The capacity of the battery is given for a specific discharge current.

Fig 5: Battery

4. MULTI STRAND WIRE: The Multi-strand wires come with a bunch of small wires. This wire bunched together to form one thick conductor. This wires are more malleable than single strand wires.

Fig 6: Multi Strand Wire

5. RESISTROR: A resistor is a 2-terminal passive electrical component that is used in the project for electrical resistance as usual. Its other applications in the proposed circuit are to reduce current flow.

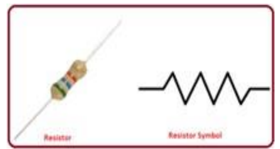

Fig 7: Resistor

6. CAPACITOR: A Capacitor is a 2-terminal passive component that has the ability to store electrostatic energy between its 2-plates.

Fig 8: Capacitor

7. BREADBOARD: A breadboard is used when printed circuit board is not created. We can change and move circuits on PCB. Breadboards are used for designing.

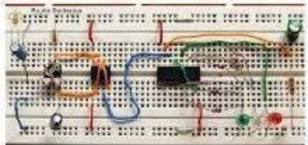

Fig 9: Breadboard

8. LED: A light-emitting diode (LED) is a semiconductor light source device which has tendency to emit light when current starts flowing through it. Electrons in the semiconductor recombine with electron and holes pair, releasing the energy in the form of photons thus emitting light.

Fig 10: LED

9. DIODE: It consists of two-terminal in which current flows in single direction. Its resistance is low in primary direction, and high in the other. In reverse bias it behaves as a conductor.

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Fig 11: Diode

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IV. METHODOLOGY

1. **Study of piezoelectric material and power generating floors**
2. **Study of flooring construction methodologies**
3. **Implementation 0f piezoelectric energy generating circuit in the flooring**
4. **Test on model**

We propose method of piezoelectric sensors to be used in implementing a power floor. Piezoelectric sensors are special type of sensors which when subjected to pressure produce AC voltage. The voltage is generated because of formation of dipoles in the material. Equal and opposite charges are deposited on opposite surface. This leads to a potential difference between the surface which is tapped as electrical energy.

The projection on the tile surface come in contact with the piezo material and hence applies force on it. The applied force produces stress inside piezo material which will produce current. There is clearance of 0.5 cm in between the springs and tile surface. The spring is provided for stability as well as protecting the piezo material from getting damage by excess load applied. **(Fig.12 & Fig.13)** shows the system model.

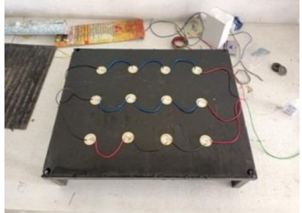

Fig.12

Fig.13

V. RESULT AND DISCUSSION

- **Calculation**

➢ **Power generating in one step**

1 step generate voltage = 6V

Current = 5 mA (from one series of sensor)

For three series of sensors = 5*3 = 15 mA

Now,

P= V*I = 6*15*10^-3

P= 90 mW

➢ **Steps require to get battery fully charged**

(Battery- 6V & 4.5 A.H)

P= 6*4.5

= 27 Watt (in 1 hr)

Table showing comparison between three different no of series of sensors attached to the tile

Table no.1 Comparison of series

Sr.No	No. of series	3	5	10
1	Power generate from one step	90 mW	0.15 W	0.3 W
2	Step require to charge battery (6V)	10,80,000	6,48,000	3,24,000
3	Steps require for one unit of electricity	40*10^6	24*10^6	12*10^6

VI. CONCLUSION

- Our aim is to produce renewable energy using piezoelectric sensors by being more interactive than other renewable energy while produce meaningful data.
- We have selected a suitable location for project. The choosen location is Shri Vadjaidevi Temple at patkhal.
- We are using piezo sensors for generating renewable energy without depleting any natural resource.
- We have connected three no of series of sensors for the tile. It requires 10,80,000 steps for battery fully charging.

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Comparative Study of Behavior of Framed Structure Under Seismic Zone III & IV Using STAAD Pro

Mr. Girish S. Gaikwad[1], Mr. Sarang P. Patankar[2], Mr. Arjun M. Shinde[3], Mr. Rohan N. Saste[4], Mr. Siddhant A. Nikam[5], Mr. A. N. Shaikh[6]
[12345]*Students, Department of Civil Engineering, YSPM's Yashoda Technical Campus, Satara*
[6]*Assistant Professor, Department of Civil Engineering, YSPM's Yashoda Technical Campus, Satara*

Abstract
Designing a structure to sustain during an earthquake makes it very uneconomical, as the earthquakes may or mostly may not occur in entire lifespan of building since it is inconsistent phenomena. In this paper a G+4 RCC building is designed in zone III and zone IV by using STAAD Pro software. Various characters like lateral displacement and storey drift will be studied. The main aim of this paper is to think on variations in RCC members, most extreme shear power, greatest redirection all these factors shows increase from zone III to zone IV.

Keywords: *Seismic zones, STAAD PRO, Lateral Displacement, .*

Date of Submission: 05-06-2022 Date of acceptance: 20-06-2022

I. INTRODUCTION

Designing structure with the help of STAAD Pro V8i which is referenced to IS 1893(PART 1): 2002 "Criteria for Earthquake Resistant Design of Structure" ensures that building has minimum strength to withstand minor earthquake occurring frequently and resisting moderate earthquakes without significant structural damage. This document is presented to improve the productivity of sustained earthquake mitigation strategies and the capacity to secure structures, frameworks, to Investigate a multiplex RCC operating for open shaking strength to think about the effects of different seismic zones, Knowing the relationship between different procedures for seismic inspection and their seismic response, gain useful learning in basic inspection, seismic assessment, drafting and identification of auxiliary parts using earthquake resistant design norms. We are also configuring the G+4 custom build, it means that if the zone changes from zone III to zone IV, the structure planned by us at that point will be fixed. Also, by calculating this we will perceive the amount spent putting together such a structure.

Seismic tremor shaking is irregular and varies with time. Be that as it may, most plan codes speak of inertia forces caused by jolting as the net effect of arbitrary jolts, such as static parallel power proportional to the structure. This strength is called the seismic design base shear VB and remains the base quantity associated with the strength-based earthquake resistant structure of structures. This strength is based on the seismic hazard in the area of the structure spoken by the seismic zone factor z. The codes reflect this by presenting a flexibility factor sa/g. This way of thinking is presented with the help of the response reduction factor r, which is larger for flexible structures and smaller for weak structures. Therefore, the seismic shake claim plan is evaluated solely on the basis of probabilistic ideas and the earthquake effects plan is called a seismic shake safe structure against reasonable estimate of interest. The design base shear VB was taken according to the Indian seismic code is 1893(part 1)-2007.

1.1 Basic Design Codes

Design should be carried so as to confirm to to the following:
1. IS 456: 2000- Plain and reinforced concrete- code of practice (fourth revision)
2. National Building Code 2005
3. Loading Standards IS 875 (Part 1-5): 1987- code of practice for design loads (other than earthquake) for buildings and structures (second revision)
Part 1: Dead Loads
Part 2: Live Loads
Part 3: Wind Loads
Part 4: Snow Loads

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Part 5: Special Loads and load combinations

1.2 Design Handbooks

SP 16: 1980- Design Aids (For RCC) to IS 456: 1978
SP 24: 1983- Explanatory handbook on IS 456:1978
SP 34: 1987- Handbooks on concrete Reinforced and Detailing.

1.3 STAAD Pro. V8i

Structural Analysis & Design is used to create the model which would then be able to investigated, analysed & designed. After examination and configuration is finished, the GUI can likewise be utilised to see outcomes graphically. It is a general useful census for auxiliary inspection and combines of Steel, concrete, Timber and aluminum construction. Its adaptability for different codes of design makes it versatile.

II. OBJECTIVES

2.1 To design G+ 4 structure for zone III & IV on STAAD Pro.
2.2 To compare the behavior of framed structure in seismic zone III & IV.
2.3 To make a total plan of the main auxiliary components of a specific structure & find out steel increment.

III. METHODOLOGY

3.1 Creation of node foci: Considering the centreline layout of the plan, we entered the hub documents into the STAAD document.
3.2 Representation of bars and segments: Using the inclusion bar layout, we plotted between beams & columns.

3.3 3D perspective on the building: Here we used the transition repetitive pattern in the Y header to get a 3D perspective on the structure.

3.4 Supports and property: After the formation of the structure, the supports at the base of the structure are specified as fixed. Likewise, the Materials were determined and the cross segments were distributed to the individuals.

4 3D render view: After feature clustering, a 3D rendering perspective can be viewed on the structure.

5 Assignment of seismic loads: We have defined the seismic loads specified in the IS1893:2002 code with appropriate ground loads in order to disable seismic loads instantly. Loads are included load case subtleties in +X, -X, +Z, -Z headings with determined seismic factor.

6 Assignment of wind loads: Wind loads are characterized according to IS 875 Part 3, depending on the determined power and input factor.

7 Assignment of dead loads: For external dividers, internal dividers, parapet dividers, constant loads including the self-weight of the structure are determined in accordance with IS 875 part 1.

8 Assignment of live loads: Live loads are relegated for each floor as 3 KN/M^2 dependent on IS 875 PART 2.

9 Adding of load combination: After all batches have been dropped, batch mixes are given with the appropriate factor of safety in accordance with IS 875 Part 5.

10 Analysis: After all the above progress paid off, we played out examination and checked for errors.

11 Design: Finally, the solid plan proceeds according to IS 456:2000, characterizing the appropriate plan orders for the various key segments. After the allocation of orders, we investigated whether there were errors again concrete design.

12 Report: After no error found the reports are downloaded and same procedure is repeated but this time with different Seismic Zone.

After following the above specifications the structure is designed for the Seismic zone III. Since, the same structure can be designed for Zone IV only with minor alterations in the Seismic Load case and reports can be compared.

IV. SIMULATION

The input data is as follow,
1.START CONCRETE DESIGN
2.CODE INDIAN
3.CLEAR 0.025 MEMB 124 125 127 TO 172 174 TO 185 189 191 195 197 TO 263 280 - 228. 281 TO 344 360 TO 424 440 TO 504 520 TO 584 229.
3.CLEAR 0.04 MEMB 81 84 88 92 93 96 97 102 112 116 TO 118 186 190 192 196 264 - 230. 265 TO 276 278 279 345 TO 359 425 TO 439 505 TO 519 585 TO 604 231.

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4.FYMAIN 415000 ALL
5.FYSEC 415000 ALL
6.MAXMAIN 32 ALL
7.MAXSEC 16 ALL
8.MINMAIN 8 ALL
9.MINSEC 8 ALL
10.RATIO 4 MEMB 81 84 88 92 93 96 97 102 112 116 TO 118 186 190 192 196 - 238. 264 TO 276 278 279 345 TO 359 425 TO 439 505 TO 519 585 TO 604 239.
11.DESIGN BEAM 124 125 127 TO 172 174 TO 185 189 191 195 197 TO 263 280 TO 344 - 240. 360 TO 424 440 TO 504 520 TO 584

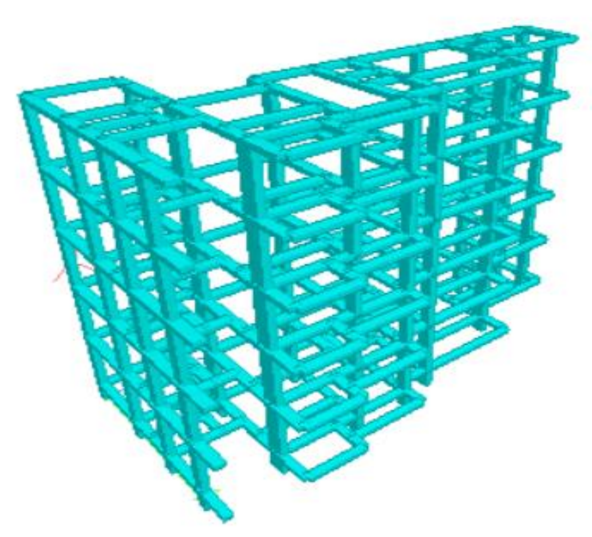

Figure1: 3-D Rendered View

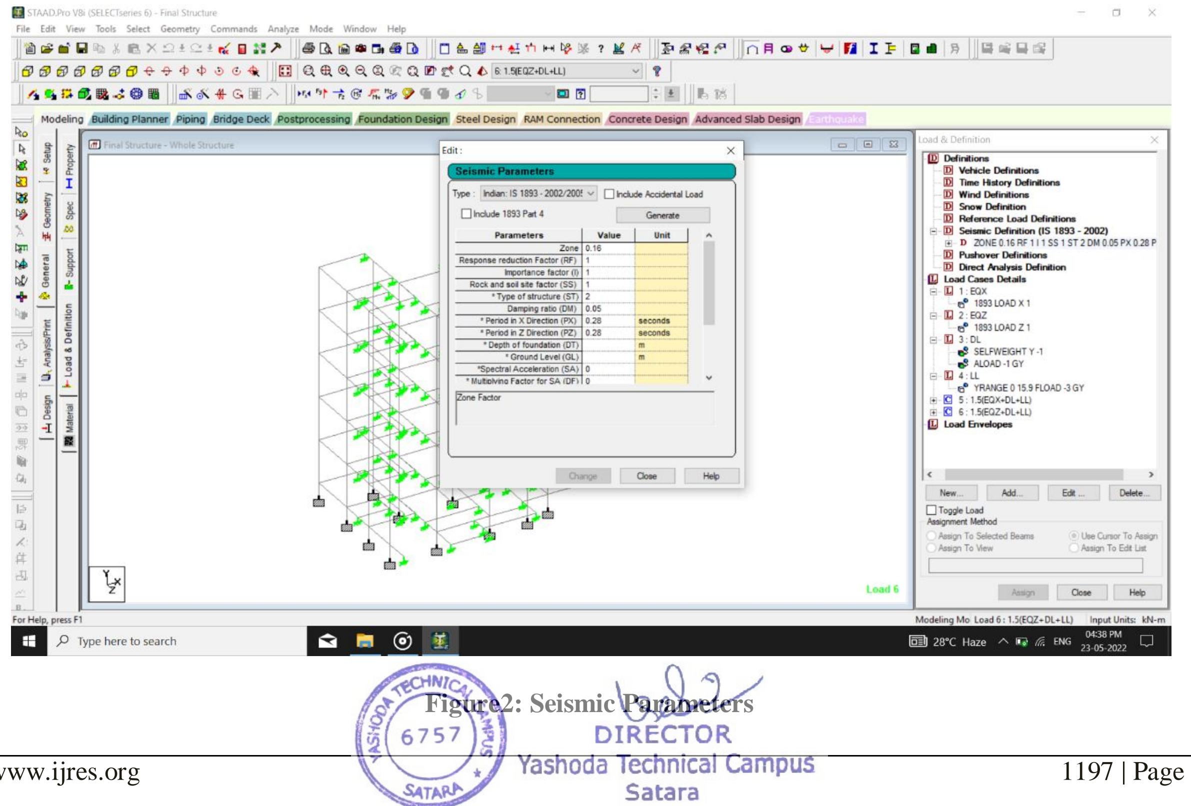

Figure2: Seismic Parameters

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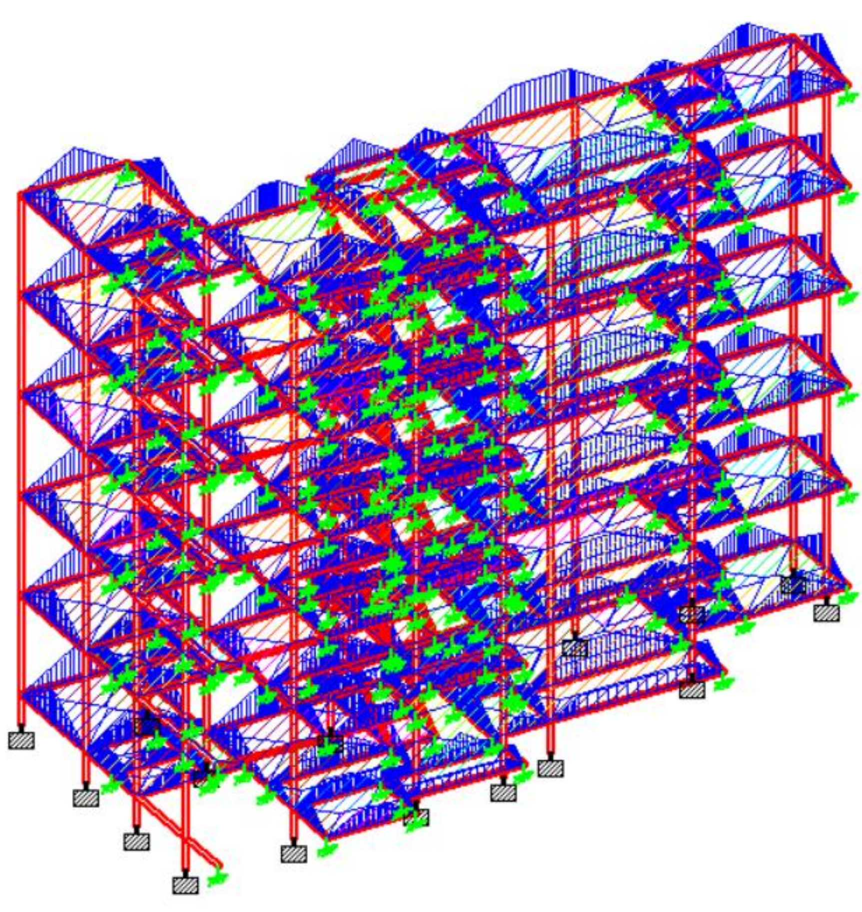

Figure3: Dead Load & Live Loads

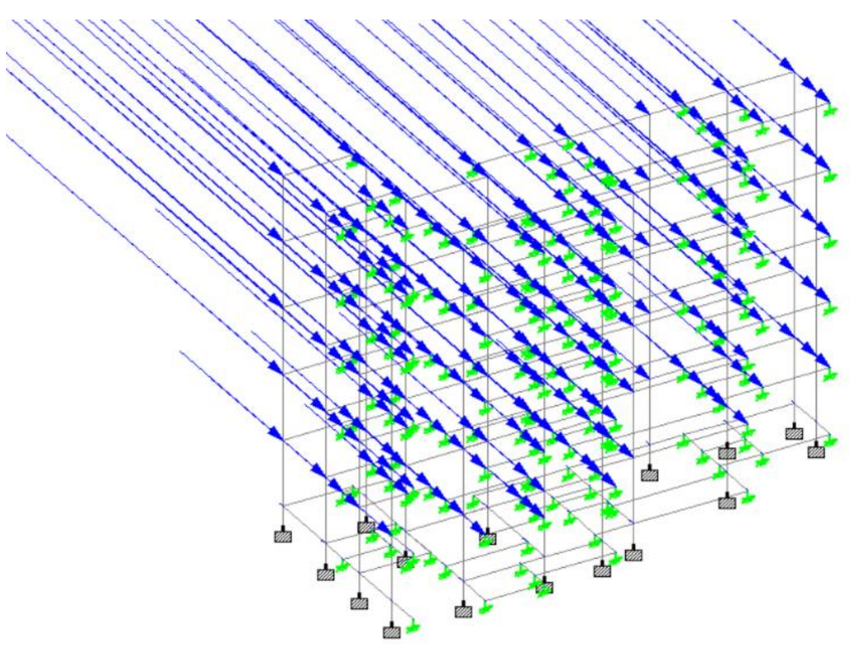

Figure4: Seismic Forces in X- Direction (maximum)

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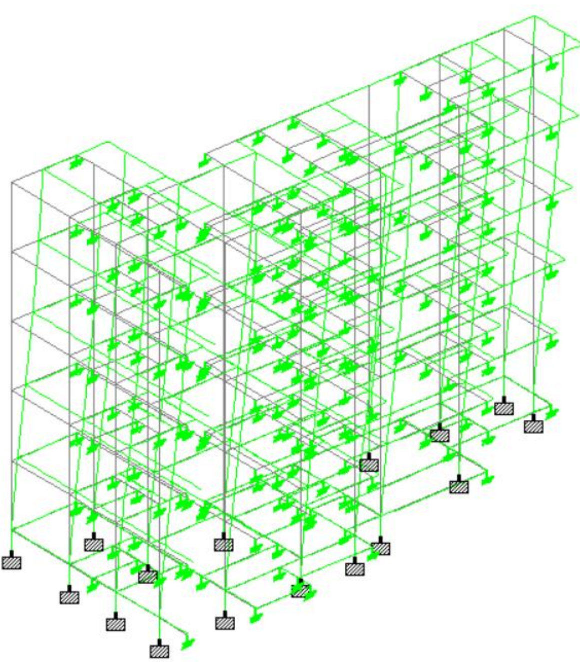

Figure5: Deflection Of Members

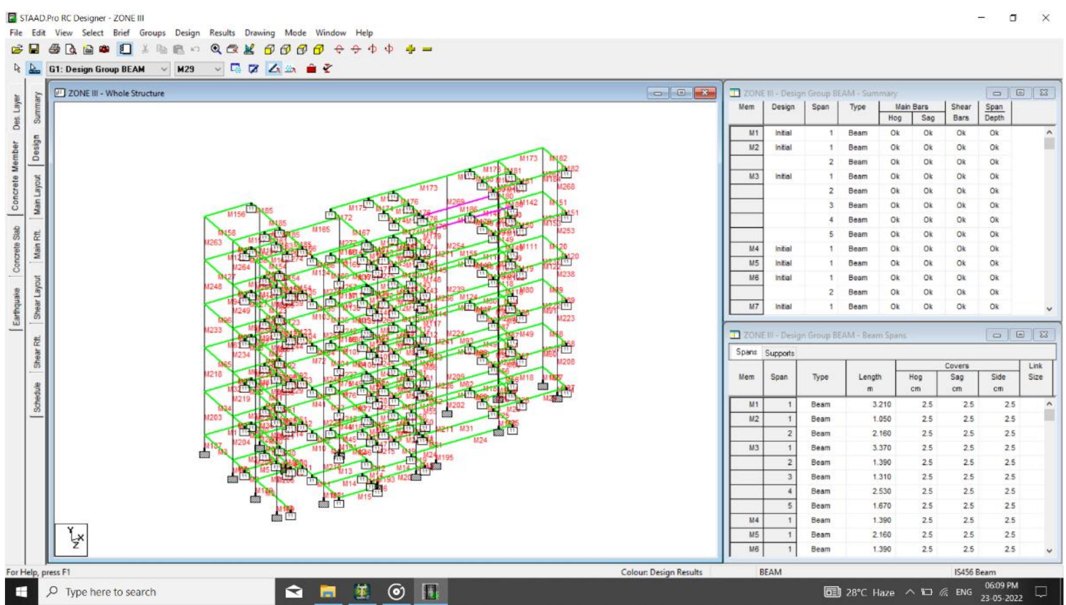

Figure6: Beam Check

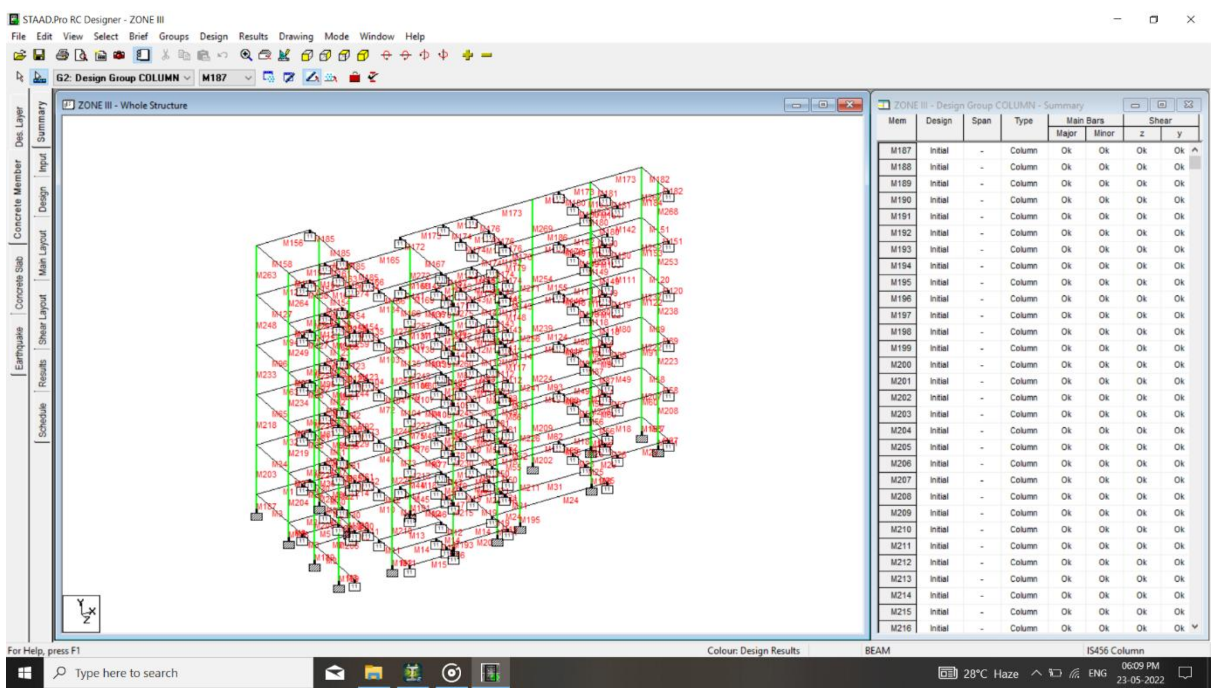

Figure7: Column Check

V. CONCLUSION

5.1 Total 2.47% more steel is require to design the structure from Zone III to Zone IV.
5.2 Maximum nodal displacement is increased by 8.33mm showing more horizontal forces in higher zone.
5.3 Maximum bending moment is increased by 30.84 kNm results in more steel in beam section.
5.4 Maximum shear forces increased by 15.32 kN resulting in additional 1.3% shear reinforcement in zone IV.
5.5 After analyzing the G+4 storey building structure, it was concluded that the building is safe under dead load, wind load and seismic loads in both zones if additional 2.5% reinforcement is provided.
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CHARACTERIZATION AND REMOVAL OF WATER HYACINTH FROM KRISHNA RIVER AT WAI TAL-WAI DIST- SATARA BY EFFECTIVE AND ECONOMIC EQUIPMENT

Prof. Lembhe Sunil S*[1], Ms. Kenjale Rutuja Vikas*[2], Ms. Nikam Shweta Anil*[3], Ms. Khatmode Pallavi Pandurang*[4], Mr. Pawar Vinayak Anil*[5], Mr. Chavan Arun Dashrath*[6], Mr. Gole Pranav Ganpat*[7]

*[1]Guide, Civil Engineering Department, Yashoda Technical Campus, Satara, Maharashtra, India.

*[2,3,4,5,6,7]Students, Civil Engineering, Yashoda Technical Campus, Satara, Maharashtra, India.

ABSTRACT

Water hyacinth (Eichhornia crassipes) is rapidly growing plant that affects aquatic plant, animals, ecosystem, navigation etc. It is also block water ways, decrease amount of dissolve oxygen. The growth rate of Eichhornia crassipes can be controlled by using chemical, biological and mechanical methods. In chemical method herbicides are use but they adverse effect on living things. Biological method is too much lengthy process & available mechanical method are very expensive. Hence the goal of our project is to develop the economical equipment to remove water hyacinth from water bodies at Ganpati ghat of wai. Equipment consist of 60&300 rpm 12 V DC motors, base frame 101cmX56cm, pvc pipes (Dia-75&140mm), bearing, shaft, conveyor belt, 12V battery, propeller.

Keywords: Water Hyacinth, Mechanical Removal, Ecosystem, Eichhornia Crassipes.

I. INTRODUCTION

Nowdays water hyacinth seems very critical problem water hyacinth is originated in the amazon basin and spread throughout the world its growth potential is very rapid as compared to any other aquatic plants its leaves are thick and ovate in shape this are 10 to 20cm across on the long stalk it has unbranched roots and also consist root cap it reproduces with both sexually as well as vegetative propagation Its profilic growth date causes blockages in water bodies it is problematic for navigation and entire aquatic life its also causes depletion of basic oxygen level in water bodies and contamination of water.

Fig 1: Water hyacinth mat at Krishna river Wai

There are some mechanical, chemical & biological methods are already available to remove water hyacinth. Mechanical method is very expensive so it is not convenient to in rural areas and chemical method consist use of chemicals has we know chemicals has adverse effects on the rest of the aquatic animal and plants as well as contaminating the water.

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DIRECTOR
Yashoda Technical Campus
Satara

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So we have developed a low cost equipment that removes water hyacinth effectively this equipment is easy to handle. It has multi purposes like with the help of air diffuser we see increase in level of dissolve oxygen in water body.

II. LITERATURE REVIEW

1. Design and fabrication of automatic water hyacinth removal and prevention machine by Gudlavalleti Deepak kumar, B Ananth Shrrecharan , T. Sai Kiran, S.S Sandeep, muli s.

In this paper they design the automatic water hyacinth and prevention machine. The machine most designed and powered by 48V DC single phase motor with speed of 270 rpm which will increase physical control of the weed. The major chain drive , rail boat, (48V), remote controller etc.

A seed sprinkler is working in this system which aids in the biological control of the machine by throwing seeds into the water bodied which prevents the water hyacinth from growing to an area.

2. Fabrication of water Hyacinth harvester by Mr. V. Shantha Moorthy, M.E., Assistant Professor, Department of Mechanical Engineering .

The aims of the project to develop a water hyacinth mechanical model which is a short term control measure. In this model harvester can be used to remove water hyacinth in the water bodies.

It is recognize that mechanical control method he then effective way of controlling the water hyacinth. This paper aimed at reducing manual work to removal the water hyacinth there by reducing labour cost involved. This decrease the blockage in water ways and continue the quality of water.

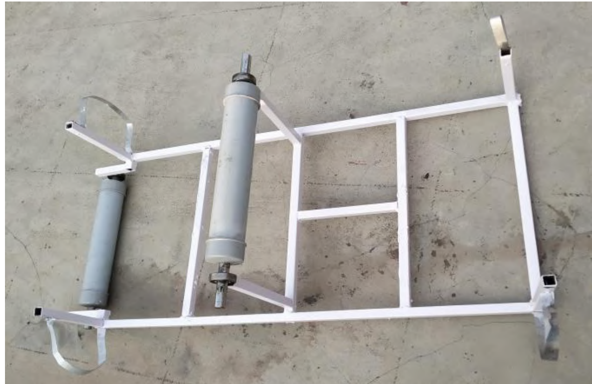
III. OBJECTIVES

1) To study the physical property of water hyacinth.
2) To take test on water (pH, acidity, alkalinity, hardness, dissolved oxygen etc.
3) To make suitable equipment from removing weed as well as cost effective.
4) To remove water hyacinth from krishna river at wai.
5) To calculate benefit cost ratio for utilization of society.

IV. COMPONENT AND SPECIFICATION

A. BASE FRAME:- A base frame is made from fabricated MS square pipes.

Specification:- Length=101cm, Width=56cm, Square pipe size=2cm x 2cm.



B. PVC PIPES :- There are two pvc pipes are used to float the equipment.

Specification:- Length=110cm, Diameter= 14cm.

C. SHAFT:- Shaft are used to transmit the power from motor to conveyor belt.

Specification:- Length=58cm, Diameter= 1.5cm.

D. MOTOR:- A DC Motor is a electrical machines that converts direct current electrical energy into mechanical energy.

Specification:- RPM=60.

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E. BALL BEARING:- A ball bearing are used to reduce rotational friction and support radial and axial loads.
Specification:- D1= 1.5cm, D2=5cm.

F. CONVEYOR BELT:- Conveyor belt are used to pull water hyacinth from river.
Specification:- Length= width=

G. COLLECTING TRAY:- The main purpose of collecting tank is to collect water hyacinth from the conveyor belt.
Specification:-

H. BATTERY :- The aim of battery to give the power to the equipment.
Specification:- 12 V 9.2 amp ups battery.

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V. METHODOLOGY

Major the volume covered by water hyacinth:

By using tape measure the volume covered by water hyacinth, (3.05Mx1.83Mx0.40m) =2.23 cubic meter.

Study the physical property of water hyacinth:

Water hyacinth has thick leaves, length of leaves is about 12 to 20 cm. plant consist of 6 to10 leaves. Height of plant is 40 to cm.

Take test of water and calculate result:

1. pH value of water sample:

1. Before to test the water sample calibration of pH meter is done with the help of standard pH solution.
2. Take 100 ml capacity beaker rinse it by distilled water dip pH electrode in distilled water.
3. Take 100 ml capacity beaker rinse it by water sample dip pH electrode in it.
4. Adjust the coarse and fine knobs to show on digital display the constant reading pH.
5. Take at least 3 readings. Calculate the mean pH value of water sample.

Fig 2: pH meter

Sr. no.	pH reading on digital display
1	6.56
2	6.52
3	6.58
	Mean = 6.55

We get pH value is 6.55.

i.e., water sample is slightly acidic in nature.

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2. Total Hardness by EDTA method:

1. For making EDTA solution we took 20 ml distilled water with 0.336 gm EDTA solution and 100 ml parent water.
2. For buffer solution 20 ml distilled water with 35 ml NH_3 and 5.49 ml NH_4Cl.
3. Take 25 ml water sample with buffer solution then add Eichrome black T- indicator after mixing this solution we get pink color by titration with EDTA solution color turns pink to sky blue.

Observation table:

Burette level	Burette reading (ml)			Constant Burette (ml)
	I	II	III	
Initial	11	11	11.6	11.20
Final	7	7	7.2	7.06

hardness ranges between 120–180 mg/lit. hence, it is moderately hard water.

3. DISSOLVED OXYGEN IN WATER SAMPLE:

1. Take a known volume of water sample in BOD (300 ml) bottle to avoid the contact of water sample with air
2. Add 2 ml of $MnSo_4$ solution in the water by using pipette. Also add 2ml of alkaline iodide-azide solution to it.
3. Shake the solution thoroughly and allow the brown precipitate of $MnO(OH)_2$ formed to settle down
4. Now add 2 ml of conc. H_2SO_4 with the help of pipette. Mix the solution again till the precipitate is completely dissolve. The characteristic brown color of iodine appears due to the liberation of iodine
5. Transfer the solution in 250 ml flask and titrate the liberated I_2 with standardized sodium thiosulphate solution until the solution becomes pale yellow.
6. Add 2 ml of starch solution, solution will turn to blue color.
7. Continue the titration till the blue color disappear.
8. Repeat the experiment to obtain concordant readings.

Observation Table:

Sr. no.	**volume of water sample**	**Burette reading**		**Volume sodium thiosulphate used**
		Initial reading	**Final reading**	
1.	**25 ml**	**0.8 ml**	**0.8 ml**	**0.025 N**
2.	**25 ml**	**0.8 ml**	**0.8 ml**	
3.	**25 ml**	**0.8 ml**	**0.8 ml**	

We get dissolved oxygen value is 1.6 ppm

i.e., dissolve oxygen range below 4 ppm

It is gravely polluted water.

Fabrication of Model:

- By using 2cm*2cm ms. sq. Pipe we made fabricated frame of size 101cm *56cm.
- Fixed a 15mm dia. Stainless steel shafts with the help of 4 bearings.
- Nylon net is use as a conveyor belt for pull out the water hyacinth.
- Two pvc pipes are fitted to base frame for floating the equipment.
- For the rotation of conveyor belt 12 V 60 rpm DC motor used.
- Installed two motors of 12V 60 rpm DC for moving equipment ahead.
- Installed 12V 9.2 Amp battery for power supply.
- The nails were fitted at the front of base frame to moving the weed.

VI. CONCLUSION

The following conclusions had been observed from the experimental results:

- The purpose of this project is to remove water hyacinth in low cost and best performance.
- This equipment is light weight so it can easily float on water & it requires less energy for running & performance will increase.

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- This method is more effective than other methods.
- As propellor rotates aeration process is done and dissolve oxygen are increase.
- As we remove water hyacinth using this equipment it increases biodiversity of aquatic ecosystem.
- It is also multipurpose to collecting floating debris, plastic bottles etc.

VII. FUTURE SCOPE

1. After completion of above project, it is noted that, this was prototype we can make such machine on large scale.
2. This machine used to remove water hyacinth in large scale.
3. Water hyacinth is produced on large scale where water is in steady state also where industrial pollution is more so there, we can use such type of machine to control water pollution and increase dissolved oxygen in water.
4. In future we can use solar panel to produce electricity for motor to run the machine.

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SUITABILITY OF RECYCLED PLASTIC WASTE IN PRODUCTION OF PAVER BLOCKS

Prof. Shah. Ajinkya.S*1, Mr. Anpat Vineet Vijay*2, Mr. Bhosale Suraj Balu*3, Mr. Botalji Suhas Suresh*4, Mr. Surve. Ajinkya. Sanjay*5, Mr. Bagwan. Saad. Salim*6, Mr. Bhosale. Pranay. Dattatray*7

*1Guide, Civil Engineering Department, Yashoda Technical Campus, Satara, Maharashtra, India.

*2,3,4,5,6,7Students, Civil Engineering, Yashoda Technical Campus, Satara, Maharashtra, India.

ABSTRACT

The purpose of our project is to make replacement for cement in paver blocks and to make cost effective than standard paver blocks. Nearly 9.46 million tone of plastic waste generates annually in India. As degradation rate of plastic waste is a slow process hence it requires more time for degradation or does not degrade. Hence our project is useful in proper managing of plastic waste in a environment friendly manner. In this project plastic waste used was of LDPE and PP type with addition of quarry dust, coarse aggregate and ceramic waste. The paver blocks were manufactured, tests performed on it and results were discussed. Sample of 180x150x50 mm size are prepared to evaluate different physical and mechanical properties, tests like water absorption test, compression test, oven test, hardness test, are carried out as per IS specifications on plastic paver blocks. The results obtained have shown good results.

Keywords: Recycle, Plastic Waste, Ceramic Waste, Paver Blocks. Shuttering Ply, Cost Effective, Compressive Test, Water Absorption Test, Hardness Test.

I. INTRODUCTION

Plastic is described as artificial or semi-artificial substances which can be polymeric and are composed of massive molecules of organics materials referred to as monomers. The large molecules that are from the during a process known as polymerization are known as polymers.

As there is a increase in population as well as urbanization and development, which is the main reason for increase in plastic waste. As the plastic waste is non-biodegradable, problem of disposal of plastic waste arises highly. To overcome this situation we can use the plastic in construction sectors as a raw material in different ways like by preparing tiles, bricks and pavements.

Paver block is aesthetically desirable in appearance, economical and has low maintenance, if properly manufactured. Recycling of plastic waste and its use in construction works gives an alternative to another materials which leads in conservation of environment.

CATEGORIES OF PLASTIC:

A. **Recyclable Plastics** (Thermoplastics): PET,HDPE, LDPE, PP, PVC, PS, etc.
B. **Non-Recyclable Plastics** (Thermoset & others): Multilayer & Laminated Plastics. Bakelite, Polycarbonate, Melamine, Nylon etc.

- **Recyclable Plastic:**

1. **LDPE (Low Density Poly Ethylene)**
2. **HDPE (High Density Poly Ethylene)**
3. **PP (Polypropylene)**

II. LITERATURE REVIEW

1. Aarti Ghude1, Ram kant2, Parv Jaiswal3, Avish Dhomne4, Akash Thool5, Sanjal Nandanwar6, Neha Ghumde7, Komal Bele8.

In this research paper mentioned above, the study on the management of plastic waste is done by using it in the manufacturing of the paver block. Recycling of plastic waste and its proper disposal is achieved in a innovative manner.

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2. **Nivetha, C. Rubiya, M. Shobana, S. Vaijayanathi, G. (2016). Production of Plastic Paver Blocks from Solid Waste.**

In this paper study on the replacement of cement by plastic waste as a binding material in manufacturing of paver blocks was done.

III. MATERIAL USED AND MIX RATIO

A. Material used for making of Paver mould –

Shuttering ply was used for making of mould instead of MS sheet, due to its light weight, easy handling, easy demoulding and cost efficiency.

B. Material used for making of Paver blocks:

- **Plastic Waste (LDPE, PP)**

Plastic waste used for making paver blocks was collected from nearby area it includes plastic bags of about 50 microns and empty cement bags.

- **Quarry Dust**

Quarry dust is a crushed sand less than 4.75mm produced from rock using state of crushing plants which was collected from the nearby quarry.

- **Coarse aggregate**

Aggregate passing via 12mm sieve and retained on 10mm sieve as per IS 383-1970 was used.

- **Ceramic waste.**

Principal waste coming from the ceramic enterprise is the ceramic waste.

C. Mix Ratio:

- **Specimen 1:** This specimen was casted by using proportion mentioned below:

(Weight of specimen = 2 Kg)

Plastic Waste = 0.5 Kg Coarse aggregate = 0.5 Kg

Ceramic waste = 0.5 Kg Quarry dust = 0.5 Kg

- **Specimen 2:** This specimen was casted using proportion mentioned below:

(Weight of specimen = 2.320 Kg)

Plastic waste = 1.250 Kg Quarry dust = 500 gm

Ceramic waste = 600 gm

- **Specimen 3:** This specimen was casted using proportion mentioned below:

(Weight of specimen = 2.430 Kg)

Plastic waste = 1.150 Kg Coarse aggregate = 300 gm

Quarry dust = 500 gm

IV. METHODOLOGY

- **Manufacturing of Paver blocks:**
- Materials used for manufacturing of pavers are LDPE, PP, Quarry dust, coarse aggregate and ceramic waste.
- **Specification of mould:**

Table No 1: Mould Specification

Material	Size of Mould	Shape of Mould	Weight of Mould
Shuttering ply	180 x 150 x 50 mm	Dumbel	1.112 Kg

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Fig No 1 : Mould.

- **Procedure of Making Plastic Paver Blocs:**

In this project, from waste plastic paver blocks were casted, following points are to be considered while casting of paver blocks. Casting of paver blocks consist of a manual casting method.

1. **Collection of Plastic:**

The plastic wastes are collection from collected from residential, commercial and constructional areas.

2. **Manual Sorting of Plastic:**

The plastic waste required for the project work was sorted from the other plastic waste manually.

3. **Melting of Plastic:**

The melting of plastic waste done manually and the source of heat was fire.

4. **Casting of Mould:**

After melting of plastic waste it was in a liquid form, which was then poured in a mould. Other materials as mentioned in mix ratio above were added to it. Then proper compaction was done to remove internal pores.

5. **Curing:**

As the casting of mould was done ,mould was kept for curing purpose for 24hrs,so that it gets harden.

6. **Demoulding:**

After curing procedure of demoulding is followed, sides of the shuttering ply mould werw removed for easy demoulding. Casted paver block was removed.

7. **Finishing:**

Finishing work of paver block was done with the help of bladed material by which the surplus material was removed and the desired shape was achieved after the proper finishing.

Fig No 2: Paver Block.

V. RESULTS AND DISCUSSION

The tests were performed to find out the quality and strength of pavers specimens. Following tests were carried out on paver specimens are as follows:

- **WATER ABSORPTION TEST (IS 10545-3: 1995 Part-3):**

The test procedure is as follows:

The paver specimens were fully immersed in water for about 24hrs. After 24hrs the specimens were removed out off the water and allowed to drain off. After that the specimens were cleaned up and the wet weight was taken, weight of every specimen was recorded and represented as (Ww) then the specimens were kept for drying purpose in natural atmosphere. Dry weight of each specimen was recorded, and is formulated by,

Water absorption = $[(W_2 - W_1)] / [W_1] \times 100$

Where,

W_1= weight of dry paver

W_2=weight of wet paver

Table No 2: Water Absorption Test

Specimen No	Area in mm²	Dry weight of specimen (W_1)	Wet Weight of Specimen (W_2)	Water Absorption in %
Specimen 1	180 x 150	2.000	2.015	**0.75%**
Specimen 2	180 x 150	2.320	2.340	**0.86%**

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Specimen 3	180 x 150	2.430	2.450	**0.82%**

- **COMPRESSION TEST – (IS 15622-2006):**

Compressive strength for plastic paver blocks of size 180x150x50 mm was obtained. The highest load at failure is taken and mean compressive strength was calculated using following equation,

Compression Strength (N/mm^2)= [Ultimate load in N / cross sectional area (mm^2)]

After 7 days of curing compressive strength obtained by block specimens is mentioned below:

Specimen No	C/S area in mm^2	Compressive Load in KN	Compressive Strength in N/mm^2
Specimen 1	180 x 150	245.25	**10.66**
Specimen 2	180 x 150	246.87	**11.51**
Specimen 3	180 x 150	313.92	**13.64**

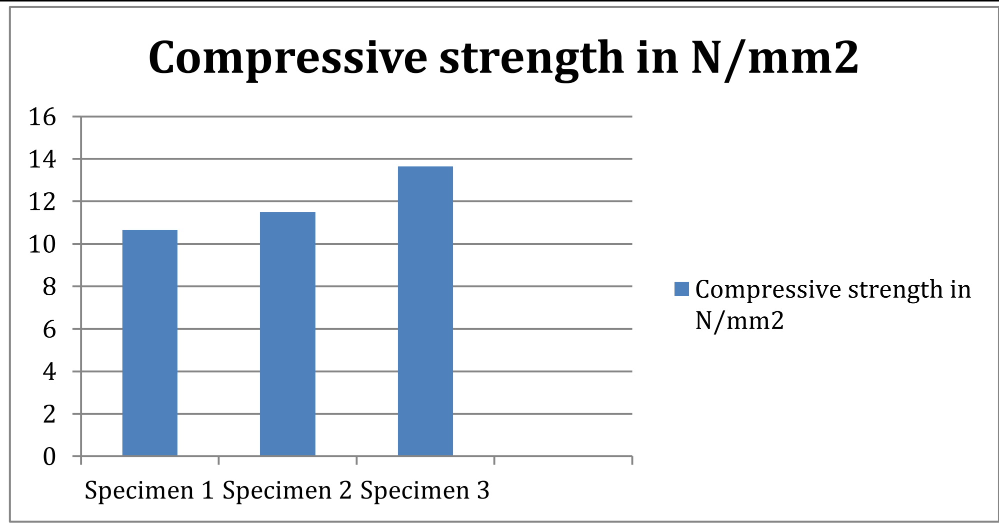

Chart No 1: Chart representing Compressive Strength of Block Specimens.

Fig No 3: CTM Machine.

- **HARDNESS TEST:**

Hardness test is carried out by making a scratch on paver block by any hard material, by which hardness of specimen was observed.

- **OVEN TEST:**

As the paver blocks have been made from plastic, to understand its melting factor through oven test method. The paver blocks have been stored in oven for 2hrs and after their conditions were noted as mentioned below:

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Table No 4: Oven Test Result

Specimen no	Temperature (^{0}C)	Remarks
Specimen 1	50	Remains same
	100	Remains same
	150	**Varies**
Specimen 2	50	Remains same
	100	Remains same
	150	**Varies**
Specimen 3	50	Remains same
	100	Remains same
	150	**Varies**

➢ **RESULT OBTAINED:**

1. Plastic paver blocks made up of waste plastic and ceramic waste gives good result.
2. Water absorption of the plastic paver blocks is **0.81%.**
3. Compressive strength obtained by CTM (compressive testing machine) on specimen is **13.64N/mm^2**
4. In oven test above 150^{0}C changes are identified in blocks due to increase in temperature.

VI. CONCLUSION AND FUTURE SCOPE

The following conclusions had been drawn from the experimental investigation:

- The usage of waste plastic in manufacturing of paver blocks have effective manner of disposal of plastic waste.
- Though the paver is light in weight it gains better compressive strength.
- Paver blocks made by the use of plastic waste, quarry dust, coarse aggregate and ceramic waste have obtained desirable results.
- Good heat resistance is observed.
- It can be utilized in non-traffic roads.
- Using of shuttering ply for making mould is more economical than MS sheet.

➢ **FUTURE SCOPE:**

- After completion of above project it is noted that the paver blocks made from plastic waste and ceramic waste shows good results after curing of 7 days period, but in future it can be done more effective and more strength can be gained by adding some another binding materials like Resin-melamine, resin polysters. All these are chemicals which can be added in future.
- If we used fire proof reagents, colour reagents for making the pavers more good in aesthetic appearance.

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To Study Effect of Gray Water on The Properties of Concrete

AKSHAY SANJAY INGALE[1], DHULDEV GULAB NARALE[2], AMOL SOPAN KOLHEKAR[3], PRANITA CHANDRAKANAT KAMBLE[4], PROF. SHAH. AJINKYA.S[5]

[1, 2, 3, 4] *Student, Civil Engineering, Satara College of Engineering and Management, Maharashtra, India.*

[5] *Guide Civil Engineering Department, Yashoda Technical Campus, Satara*

Abstract— This project aims to evaluate the potential of reused grey water in concrete and mortar in order to preserve fresh water for drinking purposes. Using both Treated Grey Water and Raw Grey Water (TGW and RGW, respectively) led to a significant increase in the initial setting time and a decrease in the concrete slump value. In addition, there was no effect on mortar soundness properties. The mortar and concrete compressive strength results obtained at 7 days moist curing time showed a significant increase. Mortar and concrete mixes using TGW cast at curing times of 28 days led to no significant effects on compressive strength.

On the contrary, the RGW achieved slightly negative impact on compressive strength at all curing ages. According to the Indian Standards, TGW and RGW are suitable for mortar and concrete production. In conclusion, TGW and RGW are potential alternatives for fresh water in the concrete manufacturing industry.

Index Terms— Drinking Water, Grey Water, Used Water, Sewage Water, Ph meter, BOD, COD, compression testing machine, M25 grade concrete.

I. INTRODUCTION

Water scarcity is the lack of sufficient available water resources to meet the demands of water usage within a region. It already affects every continent and around 2.8 billion people around the world at least one month out of every year. More than 1.2 billion people lack access to clean drinking water.

Economic water scarcity is caused by a lack of investment in infrastructure or technology to draw water from rivers, aquifers or other water sources, or insufficient human capacity to satisfy the demand for water. One quarter of the world's population is affected by economic water scarcity. Symptoms of economic water scarcity include a lack of infrastructure, causing the people without reliable access to water to have to travel long distances in or fetch water, that is often contaminated from rivers for domestic agricultural uses. Large parts of Africa suffer from economic water scarcity; developing water infrastructure in those areas could therefore help to reduce poverty. Critical conditions often arise for economically poor and politically weak communities living in already dry environment.

II. LITERATURE REVIEW

This chapter gives a comprehensive review of the work carried out by various researchers in the field of using plastic in paver blocks.

1. Lynn Schneider has been investigated “Grey water Reuse in Washington State”.(2-6-2009 - 17-9-2009):

This report summarizes the literature on the characterization of grey water by source inside of a home from on potable reuse in the State of Washington for single family homes, multi-family homes, and businesses. It summarizes available data related to the average quantity and constituents of concern associated with a variety of sources of grey water. It is meant to be used as a tool by the grey water rule advisory committee during rule development.This literature review demonstrates that the level of pollution in the total grey water stream that includes kitchen sinks, dishwashers, laundry machines used to wash dirty diapers can be equal to or greater than black water and requires regulations consistent with on-site sewage regulations. Wastewater from kitchens can be

heavily polluted with pathogens, chemicals from dish detergents, and fats, oils and grease. Wastewater from clothes washing machines used for washing soiled diapers contains increased levels of bacteria.

2. Lucy Allen Juliet, Christian-Smith, Meena and Palaniappan has studied “Overview of Grey water Reuse.(Nov18-2010):

The Potential of Grey water Systems to Aid Sustainable Water Management” Grey water, defined slightly differently in different parts of the world, generally refers to the waste water generated from household uses like bathing and washing clothes. This wastewater is distinguished from more heavily contaminated “black water” from toilets. In many utility systems around the world, grey water is combined with black water in a single domestic wastewater stream. Yet grey water can be of far higher quality than black water because of it slow level of contamination and higher potential for reuse. When grey water is reused either on site or nearby, it has the potential to reduce the demand for new water supply, reduce the energy and carbon footprint of water services, and meet a wide range of social and economic needs .In particular, the reuse of grey water can help reduce demand for more costly high-quality potable water.

III. OBJECTIVE

- To study the property of gray water.
- To study the chlorine,ph,cod,bod test on the collected sample of gray water.
- To compare the result of ph chlorination bod cod with normal water.
- To compre compressive test of concrete with variation in percentage of gray water(20% 30% 40%).

IV. MATERIAL USED AND MIX RATIO

A. Material used for making of mould –

Grey Water, Cement, Aggregate, normal water was used for making of block.

B. MIX DESIGN FOR 0.5W/C RATIO

Grade designation	M40
Type of cement	OPC 53 grade
Maximum nominal size of aggregate	20mm
Minimum cement content	300 kg/m3
Maximum water-cement ratio	0.5
Workability	100-120mm slump
Exposure condition	Moderate
Method of concrete placing	Hand placing
Degree of supervision	Good
Type of aggregate	Crushed angular
Maximum cement content	) /m3

STIPULATIONS FOR PROPORTIONING

C. SELECTION OF WATER-CEMENT RATIO

Adopt water-cement ratio as 0.50.

D. SELECTION OF WATER CONTENT

Maximum water content = 186 lt .for 20 mm aggregate

For 25-50mm slump angle=186+(6/186)=197 lt.

E. CALCULATION OF CEMENT CONTENT

Water-cement ratio Cement content =0.50 Cement content =336kg/m3

F. PROPORTION OF VOLUME OF COARSE AGGREGATE AND FINE AGGREGATECONTENT

From , volume of coarse aggregate corresponding to 20 mm size aggregate and fine aggregate (Zone I) for water-cement ratio of 0.50 = 0.60. Therefore, volume of coarse aggregate = 0.60

Volume of fine aggregate content = 1 - 0.60 =0.40

V. METHODOLOGY

- Manufacturing

Collection of sample.

Performing tests on collected water sample:- a) pH b) Chlorination c) BOD d) COD.

Analyzing test Results.

Comparing test results with potable water sample.

Collection of materials

Performing tests on materials collected.

Test to be conducted:-

a) Workability: - 1) Slump cone, 2) Compaction factor test

Casting of concrete cubes using Grey water & potable water.
Performing tests On both cubes
a)Compressive strength Test
Comparison of compressive strength at 7 days and 28 days of curing.

VI. RESULTS AND DISCUSSION

The tests are required to determine the quality and strength of specimen and therefore its suitability for the job.

- GREY WATER TEST

The test procedure is as follows:
Grey water reuse methods can range from low-cost methods such as the manual bucketing of grey water from the outlet of bathroom, to primary treatment methods that coarsely screen oils, greases and solids from the grey water before use via small systems, to more expensive secondary treatment systems that treat and disinfect the grey water to a high standard before using. Figures show some grey water treatments procedure.

PH Testing Machine

Sr. No	Parameter	Result (For GRAY water)	Result (For Normal water)	Units
1	pH	7.5	7.2	-
2	DO	2.4	6.4	Mg\lt
3	BOD	30	5.0	Mg\lt
4	COD	250	Not standard	Mg\lt

For Grey Water and Normal water

- Compression of workability by slump cone test

% of grey water	Slump (mm)
0	40
20	46
40	55
60	60

Comparison of workability by slump cone test

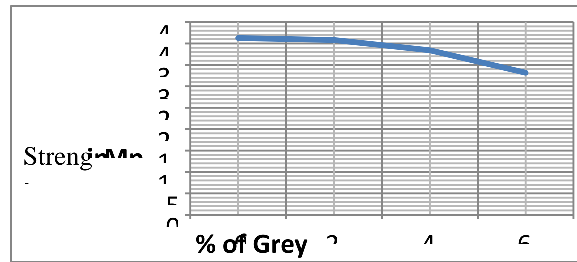
As shown in above graph we can conclude that as the percentage of grey water increases then workability of concrete also increases. . As the percentage of grey water increases by 20%, 40%, 60%, workability increases by 15%, 37.5%, 50% respectively.

Some synthetic detergents, fatty and resinous acids and their salts, alkylbenzeneulfonates are materials of air entraining admixtures and also of soaps and detergents. Therefore soapy water can improve workability.

Chart representing Comparison of compressive strength at 28 days of curing:

% of Grey water	Casting Date	Test Date	Weight (Kg)	Strength (MPa)	Mean Strength (MPa)	Peak Load (KN)
0%	27/02/21	26/03/21	9.319	40.01	40.63	900.225
			9.579	42.30		950.75
			9.230	39.60		823.5
20%	13/03/21	10/04/21	8.447	37.64	37.38	846.9
			8.485	38.10		857.25
			8.397	36.40		819
40%	14/03/21	12/04/21	8.377	36.30	35.53	816.75
			8.311	35.40		796.5
			8.401	34.90		785.25
60%	16/03/21	16/04/21	9.307	32.95	32.56	741.375
			8.107	30.30		681.75
			9.203	31.45		707.625

Results of compressive strength at 28 days of curing



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Graph of Comparison of compressive strength at 28 days of curing

CTM Machine

RESULTS OBTAINED

As shown in above graph we can conclude that as the percentage of grey water increases then compressive strength of concrete at 28 days curing goes on decreases.

When the grey water is increased by 20% , 40%, 60% then compressive strength of concrete at 28 days curing decreases by 1.1%, 6.9%, 19.40%.

CONCLUSION AND FUTURE SCOPE

- Increase in Workability: Air entraining admixtures can improve workability of concrete. Some synthetic detergents, fatty and resinous acids and their salts are materials of air entraining admixtures and of soaps and detergents both. Therefore, soapy water can improve workability. At the same time these materials are helpful for improving durability in freeze-thaw, deicer, sulphate and alkali-reactive environments.
- As we had taken the chlorinization test,PH, cod, bod test as the percentage of the gray water increases by 20% 40% 60% percent workability
- Compression stress at 28 days of curing goes on decreasing when the gray water is increased yb 20% 40% 60%
- As the conclusion we cat use the gray water more than 60% in a concrete as the strength of the concrete is decreaseing in high level.

FUTURE SCOPE

- 1 As a result of reuse, fresh water drinking supplies are conserved enabling it to remain in natural ecosystems.
- Grey water has the potential to save on average 50 per cent of an average household's water use. Apart from savings to the consumer, grey water reuse saves water authority money, reduces sewage flows and reduces the demand on potable water supplies.
- load on wastewater disposal systems is reduced and therefore their life is prolonged and capital expenditure required for upgrading and expansion is delayed, if not potentially decreased.

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Implementation of New Water Distribution Network In Village Saigaon (Rahimatpur)

Kunal Holkar[1], Dipika Mane[2], Vivek Yadav[3], Nivas Madane[4], Mrudulla Shellar[5], Shardulla Saudagar[6], Mr. Alfaj N. Shaikh[7]
1, 2, 3, 4, 5, 6, UG Students of Dept. of Civil Engineering, S.C.O.E.M. Limb, Satara, Maharashtra, India.
7Assistant Professor of Dept. of Civil Engineering, YSPM'S YTC CoE, Satara, Maharashtra, India.
Corresponding author: Kunal Holkar

Abstract
This report presents the utilization of EpaNET software within the design of the water distribution network for the village Saigaon, Satara district. the most important purpose of providing a decent distribution network is to produce sufficient pressure at each node point with less loss. A water distribution network consists of pipes, valves, tanks, etc. EpaNET could be a bug that tracks the flow of water in each pipe, the pressure at each node, and therefore the height of water in ESR. this report was accustomed do the look and hydraulic analysis of the water distribution network using EpaNET software. the tactic of distribution used here is that the combined gravity and pumping system. it absolutely was obtained that the pressure in the slightest degree junctions and flow with their velocities in the least pipes are feasible. The analysis is disbursed supported various public demands, quantities of inflows, and outflows of the overhead reservoirs. This analysis provides information about various demands, losses, and uses of the general public. the planning of a replacement network of supply will make attentive to the new demands, rate of increase within the demands. the look is formed thanks to the growth rate, and also the developing village. The report presents the hydraulic analysis of the pipeline network of Saigaon village using EPANET 2.0. for a region of 1.2 sq Km area and 1210 Population 2050. The water from the source well is taken via the network of pipes to the ESR Elevated Storage Reservoirs during the availability hours and water is supply to the world by gravity. the gap between the source well to ESR is 839 m. Simulation has been dispensed for hydraulic parameters like head pressure and flow.
Keywords: *distribution network, Epanet software, ESR tank, simulation.*

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I. INTRODUCTION

Water is that the most significant need of all living organisms. Water is employed for irrigation purposes, industrial purposes, and domestic purposes. a water distribution network should be designed in such the simplest way that it meets the demand of the increased population. An adequate installation can give better living standards. The water quality shouldn't get deteriorated within the distribution pipe. a decent water distribution network provides sufficient pressure at each point of distribution with less loss. a decent water distribution network satisfies the buyer demand at the desired time. the planning and analysis of water distribution networks may be a complex process. Water supply systems get water from a range of locations, including groundwater, surface water. The water is then, in most cases purified, disinfected through chlorination, and sometimes fluoridated. Treated water either flows by gravity or is pumped to a reservoir, which might be elevated like a reservoir or on the bottom. The water is then fed into the distribution. Water distribution system, hydraulic infrastructure contains elements like pipeline, tanks, basin, pumps, and valve, etc. is important to produce water to the consumer's elements of a distribution system include distribution mains, arterial mains, storage basin and system elements (valves, hydrants, mainline meters, service connections, and backflow preventers). Distribution main are the pipes that compose the distribution complex. Their purpose is to transmit water from water sources or treatment work to users. Service connection that connects either other plumbing systems or a private building to distribution system mains. The water distribution system consists of an interconnected series of pipelines storage facilities and elements that convey waters that are used for drinking and also meeting the hearth protection needs for cities, schools, homes, hospitals, industries, businesses, and other facilities.

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II. LITERATURE REVIEW

Jagtap R. et all [1], This study is based on assessment of existing water distribution network using EPANET 2.0 software. The pipe network and junction network system is simulated to understand its behavior for different inputs using EPANET 2.0. Simulation has been carried out for hydraulic parameters such as head, pressure and flow rate. The results obtained verify that the pressures at all junctions and the flows with their velocities at all pipes are feasible enough to provide adequate water by the network of the study area.

G. ANISHA et all [2], This research is all about the analysis of the existing network and concludes about the reliability on the network for the future. The analysis is carried out based on various public demands, quantities of inflows and out flows of the over-head reservoirs. This analysis provides the information about various demands, losses, and uses of the public. The design of a new network of supply will make the municipality be aware of the new demands, rate of increase in the demands. The design is made keeping in view of the population growth rate, and the developing town. The design brings out an improvement in the existing network.

Dr. G. Venkata Ramana et all [3], This paper highlights only the effective design and distribution of network of pipes using EPANET tool. The residual head at each and every node was found out by having the elevation as input and thereby the corresponding flow quantities were derived like residual head, velocity and nodal demand etc.

III. OBJECTIVE

1) To design a water distribution network that can supply an adequate quantity of water to the consumer's end with sufficient pressure and 24*7 supply.

IV. METHODOLOGY

- Study area.
- Population forecasting.
- ESR capacity calculation.
- Analysis of distribution network with Epanet software.

4.1 Study Area.

Saigaon Gram panchayat is located in Koregoan tehsil of Satara district. Saigaon gram panchayat has an area of 1.3 sq. Km. Saigaon is located at a distance of 17 km to the southeast of Satara and 14 km south of Koregaon. The study area covers 7 zones of Saigaon village and some eastern parts of Dhamner village attached to Saigaon village. The current population of the study area is 840.

Table 4.1.1 Study Area Basic Information

The current population of study area	840
Total no of households in study area	150
No of shops in area	08
No of primary schools in area	01

4.2. Population Forecasting

Methods of population forecasting.

a. Arithmetical increase method.
b. Geometrical increase method.
c. Incremental increase method.

As Saigaon is a small village but the migration rate of population from Karnataka as daily wedge labor is more here. These migrated people work on construction sites or in MIDC AREA near Satara. So here we adopting a method for population forecasting is the "**Incremental Increase Method**".

The design period for proposed water distribution supply scheme is 30 years. so population forecasting have to be done for the year 2050.

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Table 4.2.1 Past Increase in Population

Year	Population	Arithmetical Increase in Population	Incremental Increase in Population
1990	500		
2000	610	110	
2010	720	110	0
2020	840	120	10
		Avg. d=113.3	Avg. t=5

Table 4.2.2 Forecasted Population with Incremental Increase Method

Year	Forecasted Population
2030	959
2040	1082
2050	1210

4.3 ESR capacity calculation.

Water supply or distribution reservoirs are hydraulically an integral part of the water distribution system, and the proportion of water supply pipes leading to them is usually so high that they can supply water at a rate high enough to meet with the maximum 24 hours a day. Hourly demand that exceeds this ratio is supplied by storage.

Table 4.3.1 Total Daily Demand

Forecasted Population	Supply	Total Requirement
1210	70	84700 LTRS

Table 4.3.2 Average Hourly Demand

Total Demand	Supply Hours	Per Hour Demand
84700	24	3529.17 Ltrs

Table 4.3.3 Modified mass diagram for 70 LPCD supply

Start time	End time	hourly demand factor	cumulative demand (Ltrs)	cumulative pumping (Ltrs)	cumulative surplus or deficit (Ltrs)
0	1	0.20	705.83	0.0	-705.8
1	2	0.20	1411.67	0.0	-1411.7
2	3	0.20	2117.50	0.0	-2117.5
3	4	0.20	2823.33	0.0	-2823.3
4	5	0.40	4235.00	0.0	-4235.0
5	6	0.80	7058.33	0.0	-7058.3
6	7	2.25	14998.96	0.0	-14999.0
7	8	2.25	22939.59	0.0	-22939.6
8	9	2.25	30880.21	0.0	-30880.2
9	10	2.25	38820.84	10587.5	-28233.3
10	11	1.00	42350.00	21175.0	-21175.0
11	12	1.00	45879.17	31762.5	-14116.7
12	13	0.60	47996.67	42350.0	-5646.7
13	14	2.25	55937.30	52937.5	-2999.8
14	15	0.70	58407.71	63525.0	5117.3
15	16	0.70	60878.13	74112.5	13234.4
16	17	0.70	63348.55	84700.0	21351.5
17	18	2.25	71289.17	84700.0	13410.8
18	19	0.90	74465.42	84700.0	10234.6
19	20	0.90	77641.67	84700.0	7058.3
20	21	0.70	80112.09	84700.0	4587.9
21	22	0.70	82582.51	84700.0	2117.5
22	23	0.40	83994.17	84700.0	705.8
23	24	0.20	84700.01	84700.0	0.0
	maximum surplus			21351.5	
	maximum deficit			30880.2	
	ESR capacity required			52231.6	

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Table 4.3.4 Design ESR Capacity

ESR min required	52231.6 ltrs
Reserve for breakdown (30 LPCD)	37769 ltrs
Firefighting reserve	10000 ltrs

So design ESR capacity is 100000 ltrs (1 lakh liters)

4.4 Distribution Network Analysis with Epanet 2.0

We are designing distribution network for next 30 years for the village Saigaon. We are going to use here HDPE pipe which has coefficient of roughness 150 and durability up to 50 years.

❖ **Distribution network design**

1. Gravity based distribution supply of water.
2. Dead end pipe network.
3. 27 x 7 supply

EpaNET 2.0

For the analysis of distribution network, we are going to use EpaNET 2.0 software. EpaNET is a computer program that performs extended period simulation of hydraulic and water quality behaviour within pressurized pipes.

Figure 4.4.1 Saigaon Village Satellite Image

Figure 4.4.2 Saigaon Village

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Epanet analysis report

Network Table - Nodes at 9:00 Hrs

	ELEVATION	BASE DEMAND	DEMAND	HEAD	PRESSURE
Node ID	M	LPM	LPM	M	M
Junc n165	23.68	0.39	0.88	38.7	15
Junc n166	21.58	0.39	0.88	38.6	17.06
Junc n167	23.42	0.39	0.88	38.7	15.24
Junc n168	21.99	0.39	0.39	38.7	16.68
Junc n169	21.58	0.39	0.39	38.7	17.08
Junc n170	19.32	0.39	0.88	38.7	19.34
Junc n171	19.2	0.39	0.88	38.7	19.46
Junc n172	19.07	0.39	0.88	38.7	19.59
Junc n173	18.59	0.39	0.88	38.7	20.08
Junc n175	22.4	0.39	0.88	38.7	16.26
Junc n176	21.8	0.39	0.88	38.7	16.86
Junc n177	20.3	0.39	0.88	38.7	18.36
Junc n178	19.4	0.39	0.88	38.7	19.25
Junc n179	18.36	0.39	0.88	38.7	20.3
Junc n180	23.31	0.39	0.88	38.7	15.34
Junc n181	21.2	0.39	0.88	38.7	17.45
Junc n182	19.1	0.39	0.88	38.7	19.55
Junc n183	18.18	0.39	0.88	38.7	20.47
Junc n184	22.25	0.39	0.88	38.6	16.39
Junc n185	20.86	0.39	0.88	38.6	17.79
Junc n186	19.08	0.39	0.88	38.6	19.57
Junc n187	17.32	0.39	0.39	38.6	21.32
Junc n188	19.58	0.39	0.88	38.6	19.06
Junc n189	15.17	0.39	0.39	38.6	23.47
well	0	N/A	-251	0	0
ESR1	32.68	N/A	183	38.7	6

V. RESULTS AND DISCUSSION

The water distribution network has been designed and analyzed successfully using the EpaNET software. At the end of the analysis it was found that the resulting pressure at all junctions and the flows with their velocities at all pipes are adequate enough provide water to the proposed area.

In the analysis its found that residual pressure is greater than 15 m at each node. And IS 1172 recommends minimum residual pressure of 7m, so end user will get water with sufficient pressure.

Assumed internal diameter of 90 mm is sufficient to withstand for the pressure for the entire network.

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Analysis and Design of Sand Filter by using Capped Coconut Shell and Coal

Amey Ramesh Shinde[1*], Deepa Yashwant Gosavi[2], Shivani Padmakar Jadhav[3],
Sudarshan Sanjay Chougule[4], Dnyaneshwari Prashant Kamane[5], Alfaj N. Shaikh[6]
[1,2,3,4,5]*UG Student, Department of Civil Engineering, YSPM's YTC College of Engineering, Satara, India*
[6]*Assistant Professor, Department of Civil Engineering, YSPM's YTC College of Engineering, Satara, India*

***Abstract*: A study was done to determine about sand filter these filters are commonly used in conventional water treatment plants. Problems like mud ball formation and unsatisfactory effluents are affecting sand filter beds. It has been found out that dual media and multimedia filters can overcome the limitations of sand filter. Capping materials such as crushed coconut shell and coal is used as a dual media. Design dual media filters capped with crushed coconut shell proves to be more efficient, economical and durable. Three models having capacity of 10 liter are practiced. All these models consist layers of gravel, sand and capping material of thickness 7cm each. Crushed coconut shell and coal are used as capping material in different models respectively. The water sample was collected from nearby river. The tests which are conducted on sample are pH, Temperature, Total Dissolved Solids, Alkalinity, and Turbidity. The efficiency is based on test results.**

***Keywords*: Sand filter, Coconut shell, Coal, Turbidity, pH, TDS.**

1. Introduction

Water is a basic need of human being; hence the provisions of clean water is an important issue to solve [1].

From hundreds of years there is a technology called slow sand filtration which is used for purpose of the drinking water. This process is well-suited for small, rural areas since it does not need a high degree of operator skill. As its name implies, slow sand filtration is used to filter water at very slow rates. The typical filtration rate is at least fifty times slower than for rapid rate filtration [7]. Due to slower rates of filtrate water, a large area is required for the filtration. Chemicals are not needed for proper filtration operation. Removal of particles is completed primarily through biological processes that provide treatment. The biological activity is located primarily in the top surface of the filter known as the Schmutz decked, although recent research has stated that biological processes throughout the depth of the filter bed may also influence particle removal. A ripening period from several weeks to several months is necessary [7]. So that the biological organisms mature in a new slow sand filter. Purification is always a need from the ancient times [7]. Risk of pollutants should be reduced from. So, the government are taking many efforts to provide adequate and safe drinking water to society by constructing water treatment plants in India. In India sand filter are mostly used to remove the suspended and colloidal particles from water in filtration process for the faster rate. Capping of existing sand filter is the promising method of improving the performance of sand filter [1]. Water purification or drinking water treatment, is the process of removing contaminants from surface water to make it safe and potable for human consumption. We all know that access to clean, fresh water is fundamental to our health and well-being. Most water filters remove harmful Chemicals and bacteria which if consumed can cause diseases.

2. Literature Review

Teena Ann Thomas and K. Mophin Kani. Slow sand filtration is a technology that has been used for potable water filtration for hundreds of years. It is a process well-suited for small, rural communities since it does not require a high degree of operator skill. As its name is, SSF is used to filter water. This water filtrates at very slow rate. It is observed that the typical filtration rate is at least fifty times slower. Due to this slow rate of filtration, a large land area is required for the filtration basins. There is no need of chemical required for proper filtration operation. Removal of particles is completed primarily through biological processes. These also provide treatment. The biological activity is located primarily in the top surface of the filter known as the “schmutzdecke,” Recent studies has stated these biological processes throughout the depth of the filter bed may also influence removal of particle. A “ripening” period from several weeks to several months is necessary for the biological organisms to mature in a new slow sand filter.

Mah, PFRA Canada-Saskatchewan Agri-Food Innovation Fund (AFIF). June 2001. Slow sand filtration has been used successfully in Europe since the early 1900s, and is still a popular method of treating municipal water supplies. Research, and other observations, show that slow sand filtration can effectively remove cysts and coli form bacteria from raw water, and is an innovative, cost effective, low maintenance treatment process. This system works best as part of a multi-barrier treatment approach. A slow sand filter is comprised of a bed of graded sand which is supported by a layer of gravel. This filter media is confined in a box with openings at both ends m allowing water to flow in and out, while operating on a top-

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down, gravity basis. The filtration process a form of natural, biological water treatment is used to remove solids, precipitates, turbidity (muddiness) and in some cases bacterial particles that produce bad taste and odour. Slow sand filtration is well-suited to treat farm water supplies that have reasonable raw water quality.

Sudhir Kapgate, Amol G. Gore, Gaurao S. Kale, Sagar I. Wanjar, Kunal P. Wanode, Rushikesh B. Balpande. Rainwater is an important source to feed the groundwater aquifer, which is done directly or by harvesting and recharging. Purification is always a need from the ancient age of civilization. So the central and state government are taking effort to provide adequate and safe drinking water to society by constructing water treatment plants in India. In India sand filter are mostly used to remove the suspended and colloidal particles from water in filtration process for the faster rate by setting out the different sand beds in constructing it. Designing 'Dual media filter capped with crushed coconut shells' proves to be more efficient, economical and durable.

Abdol Majid Fadaei Department of Environmental Health Engineering, School of Health, Shahrekord University of Medical Sciences, Shahrekord, Iran. Access to safe drinking water has been an important national goal in rural area and other areas. Slow sand filtration (SSF) is one of the oldest water treatment processes used to produce microbiologically safe drinking water. It is shown that safe drinking water was achieved by a combination of a protected and high quality source at the initial point and maintaining quality from the initial supply (source) point through to final consumption. Our study showed efficiency of physical and biological treatment of slow sand filter was relatively desirable but, for water quality improvement it is suggested that a chemistry and microbiology lab in treatment plant be set up. Also filter washing and cleaning should be accomplished on time.

3. Objective of the Project

If you have a Table, simply paste it in the box provided below and adjust the table or the box. If you adjust the box, you can keep the tabl Based on the literature survey the following aims and objectives were decided.

- To construct, design and test the model of slow sand filter capped with coconut shell and slow sand filter capped with coal.
- To compare the performance of slow sand filter capped with coconut shell and slow sand filter capped with coal on the basis of quality of effluent produced which is measured in terms of parameters such as pH, total dissolved solids, alkalinity, hardness, chlorides and turbidity.
- To study and design a sand filter for the removal of pathogen and suspended solids from water using coconut shells, anthracite coal, river sand and gravel for rural area.
- To improve water quality and reduce the cost of providing the clean water and improve ecosystem by maintaining water quality to acceptable levels.
- Improving water system efficiency and resource conservation.

4. Materials and Methodology

In this Paper testing facility, experimental procedures and experimental programs are included. Design of experimental setup is done based on the basic design of sand filter.

A. *Material Specification*

The materials used for filters are gravel, sand, coconut shell and coal.

Table 1
Materials and specifications

S. No.	Material	Particle Size
1.	River Sand	0.35-0.60mm
2.	Coal	2-3cm
3.	Gravels	4-5cm

1) *Gravel*

Gravel which retained on 4.75mm has been used as supporting media for sand layer [3]. The depth of gravel layer in the filtration units is 20cm. Gravel was washed and ovendried thoroughly before using as the supporting filter media layer [3].

2) *Sand*

River sand having uniformity co-efficient 1.7 and effective size 0.60mm is used as filter material [3]. Sand was washed with clean, sun dried and oven dried before using as filter media. The depth of sand layer maintained in the filtration unit is 7cm.

3) *Coal*

The burned wooden coal is used as capping material above sand layer. The depth of coal layer maintained in the filtration unit is 7cm. The coal is washed and the blackness of the coal is removed and then placed in filter. The strong quality of coal is to decolorize and remove taste from water. With an abundance of larger pores, it attracts certain dissolved chemicals and removes larger particulates. Wastewater treatment plants use it to filter water, while the food industry often uses it to decolorize juices, liquors and other products. Coal is a natural and non-toxic material. The charcoal not only removes impurities it also adds some important minerals such as calcium, magnesium and iron to improve the water quality.

B. *Design of Model*

We will prepare three models of filters which will have cylindrical shape. The first model will have gravel as a base material, sand as filter media above it and capping of coconut shell at the top. The second model will also contain gravel as a base material but the coal will be used as a capping material between gravel and sand. The third model will again contain gravel as a base material and coal and coconut will be used as a capping material between gravel and sand. All three models will have storage of same capacity and an outlet. So basically, all three models will contain layers of gravel, sand, coconut shells and coal and has drainage attached between top part and bottom i.e. storage. The capacity of the filter is 40 liter in which top part has 30-liter capacity and the storage has 10-liter capacity overall diameter of the filter is 27cm.The fig. 2.B.1

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experimental setup of slow sand filter with coconut capping and coal.

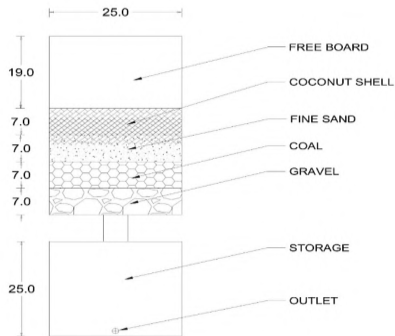

Fig. 1. B.1 schematic model

C. Methodology

In this section, testing facilities, experimental procedures and experimental programs are included. Design of experimental set up is done based on the basic design of slow sand filtration. As per the literature review the design for set-up is done. The following procedure was adopted for conducting the test:

1. Filter layer consisting of gravel bed of 7 cm thickness, sand layer of 7cm thickness and coconut shell layer of 7cm thickness was spread in the filter unit.
2. The water obtained from the lake was stored in a large container for a detention period of about 3-4 hours.
3. Influent water is fed into the filter with the help of a collector of 10 liters capacity has been placed well above the filter unit.
4. A head of water above the filter media in the filtration unit of 19cm was maintained throughout the test period. The raw water was fed to filtration unit continuously through dispenser placed above the filtration unit.
5. Influent and effluent samples were taken at a frequency of every 2 hours. These samples are tested for pH and turbidity. The experiment has been carried out up to 24 hours.

5. Experimental Work

Following test are conducted on water sample**:**

- Temperature Test
- pH Test
- Turbidity Test
- Total Dissolved Solids
- Hardness Test

6. Results

Table 2

Tests result of outlet of SSF capped with coal and coconut shell.

Parameters	Hardness	Ph	TDS	Turbidity	Temp 0^C
1	44.8	7	104.5	3.30	16
2	43.7	7.1	103.9	2.91	16
3	44.2	7.3	104	3.26	17
4	45.1	7.35	105.8	3.45	18
5	44.9	7.46	104.7	2.99	15
6	46.2	7.5	106	3.01	17
7	45	7.56	105.2	3.42	16

7. Conclusion

The following conclusions can be drawn from the project study on different aspects of slow sand filtration.

- Slow sand filters capped with coal having better removal efficiency of Hardness, Total dissolved solids comparing with slow sand filter capped with coconut shell.
- Turbidity and colour removal efficiency decline considerably with higher filtration rates, although the filtrate quality remains reasonably good.
- With less flow rate and the periodic replacement of filter media are the major limitations of the slow sand filters.
- Slow sand filters capped with coconut shell and coal together have shown effective removal of turbidity, Total dissolved solids comparing with slow sand filter capped with coconut shell and slow sand filter capped with coal.

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Utilization of Press mud for Improvement of Strength of Interlocking Bricks

Mr. Shaikh. A. N.[1], Miss.Deshmukh P.S.[2], Mr. Shelar V.E.[3]

[1,2,3] Asst. Professor, Department of Civil Engineering, Dnyanshree Institute of Engineering and Technology,Sajjangad Satara, Maharashtra, India.

Abstract -Bricks are considered to be the most widely used and useful material of construction all over the world. In recent years, Interlocking brick has made significant advances which have resulted in economical improvements in strength of clay bricks. The economic development of nation depends upon the intelligent use of locally available materials. To overcome the use of natural resources and by attempting to use waste materials like waste Press-Mud and waste Fly ash in bricks could result in low cost construction. Waste Press mud is the byproduct of sugarcane factory which causes environmental problems because of its high production and less use. Sugar industries produce the huge amount of press mud and other waste materials. The production of press mud is significantly increased, due to increasing of the production of sugar and increasing the generation of new sugar factories also the use of waste press mud in manufacturing of interlocking bricks could help to avoid the problems related to environment pollution. An attempt has been made in this study to determine the maximum compressive strength of interlocking brick by using press mud consisting of sugarcane waste as partial replacement for fine aggregates (Grit). By using press mud, we can reduce the self-weight of brick. Interlocking bricks are used for easy construction of mortar less masonry and better appearance.

Key Words: Sugarcane Waste, Press Mud, Interlocking Brick, Mortar, Fly Ash, Grit.

1. INTRODUCTION

There is a strong demand for environmentally safe reuse and effective disposal method sugarcane press mud due to the increasing amount of sludge generated by various industries and plants in India. Landfills are commonly used for disposal of sludge in India, rapid urbanization has made it increasingly difficult to find suitable landfill sites. Therefore, incineration has become one of the few alternatives available for disposal of sugarcane press mud. The ultimate disposal of incinerated press mud can be accomplished by using it as engineering construction material. One possible solution for the management of this sugarcane press mud is to re-use it as a building material, namely, to incorporate this sugarcane waste press mud into interlocking bricks. The cement interlocking brick is a one of the most useful masonry building materials. The recycling of waste materials by incorporating them into interlocking bricks has been a popular topic of investigation over the last century, with varying degrees of success across a wide range of waste material of sugarcane press mud. This popularity is likely due to flexibility on the type of wastes which can be mixed into the brick making material, but more importantly, the high temperature involved in firing the bricks allows for the volatilization of dangerous Component, as well as the fixation of wastes into the vitreous phase of the brick. The current study investigates the potential for reusing sugarcane press mud by using it as a partial replacement of material [1].

1.1 What is Sugarcane Press Mud?

India is the second largest producer of the sugar in the world, with an annual output of 25 million tonnes. Among the steps leading to the production of refined sugar is the separation of sugarcane juice from the associated particulates. Upon this separation a solid residue is obtained which is called the press mud. In a typical sugar factory, the processing of 100 tonnes of sugarcane produces about 3 tonnes of press mud. In Maharashtra some sugar factory's 8–10 million tonnes of press mud are generated annually [2].

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1.2 COMPOSITION OF PRESS MUD:

Press mud from the sugar industries is a very useful source of fertilizer as well as some substances. The major use that has recently been developed in India is in bio composting (usually trade named as Bio earth) where it is treated with the spent wash from the distillery. The composition of press mud is given in Table-1. Its usefulness as fertilizer is based on the nutrient content of the press mud and the spent wash as shown below: [2].

Table -1: Composition of press mud.

Sr. No.	Composition	(%)
1	Crude wax	5-14
2	Fiber	15-30
3	Crude protein	5-15
4	SiO	4-10
5	CaO	1-4
6	PO	1-3
7	MgO	0.5-1.5
8	Total ash	9-10

Table -2: Nutrientcontentsof press mud.

Composition	Press mud	Spent wash (mg/l)
Nitrogen	1.15 – 3.0	2630
Phosphorus	0.60 – 3.50	201
Potassium	0.30 – 1.80	222

1.3 GENERATION OF PRESS MUD:

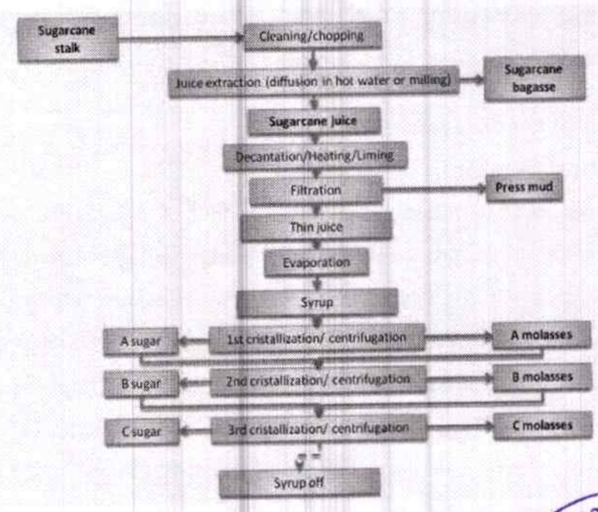

Fig -1: Generation of press mud.

2. LITERATURE REVIEW:

The waste from the industries is very harmful for the environment and also to our health, if not disposed in proper manner. The solid residue of sugarcane after crushing, extraction of its juice and before crystallization of s ugar.is known as “press mud”. India is one of the largest agriculture residues in the world [2]. The one way to dispose this waste is its use as fertilizer. But this is suitable for particular crops only. So, farmers avoid using it. The use of Sugarcane waste in brick can save the sugarcane industry disposal costs and produce an ecofriendly brick for construction. Sugarcane crop cultivation in India forms an important part of the Indian agricultural economy. The press mud can be used to recover protein, sugar and wax from press mud.

3. METHODOLOGY:

For the analysis purpose various interlocking brick samples are casted as per mix design with different percentage of sugarcane press mud and. the whole analysis is done in eight step which is given below.

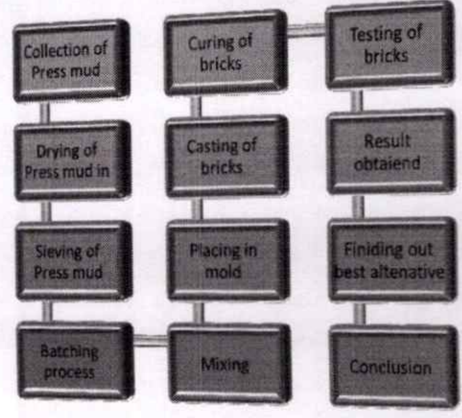

Chart -1: Process chart

3.1 COLLECTION OF PRESS MUD FROM FACTORY SITE:

Fig -2: Collection of press mud

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3.2 DRYING OF PRESS MUD IN SUN:

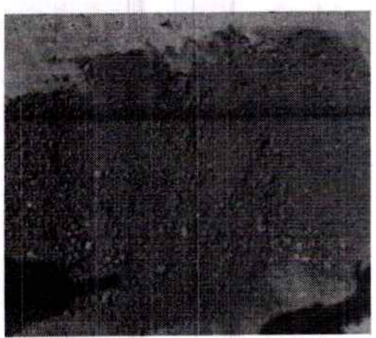

Fig -3: Drying of press mud

3.3 CASTING OF BRICKS:

Fig -4: Casting of bricks

3.4 CURING OF BRICKS:

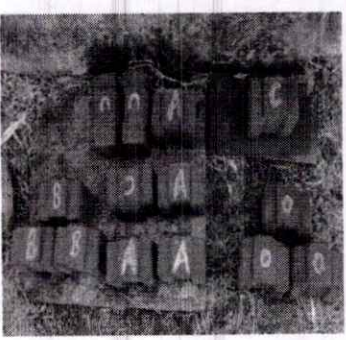

Fig -5: Placed for Curing

4. TEST ON BRICKS:

4.1. SHAPE AND SIZE TEST:

In this test, a brick specimen is closely inspected. It should be of standard size and its shape should be correctly rectangular with sharp edges. For this test, 3 bricks are selected at random and they are stacked length wise, along the width and along the height.

Results observed are:

1. One brick has not proper sharp edge.
2. Shape of brick slightly change due to breaking of edges.

4.2 WATER ABSORPTION TEST:

A brick is taken and it is weighted when it is dry. It is then immersed in water for a period of 24 hours. The brick is weighed again. The difference in weight indicates the amount of water absorbed by the brick. It should not exceed 20 percent of weight of dry brick.

Table -3: Water Absorption Test Result

Sr. No.	Block Name	Water absorption (%)
1	O	17.50 %
2	A	20.50 %
3	B	22.83 %
4	C	26.00 %

4.2 COMPRESSIVE STRENGTH TEST:

In this test the brick specimens are immersed in water for 24 hours. The specimen O, A, B, C is placed in compression testing machine. Then the load is applied axially at a uniform rate of 10 N/mm^2.The load and strength are noted accordingly.

Table -4: Compressive Strength Test Result

Sr. No.	Block Name	Compressive strength N/mm^2
1	O	3.54
2	A	4.16
3	B	3.75
4	C	2.93

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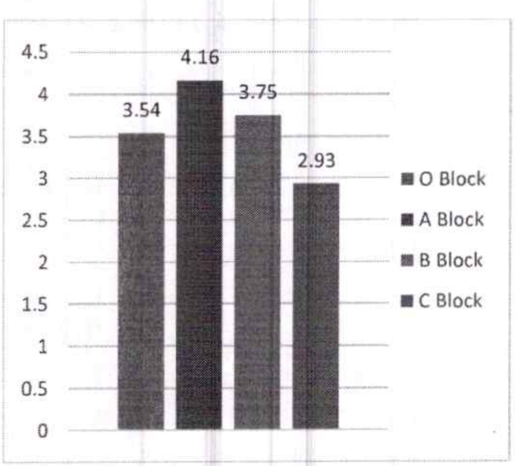

Chart No.2: Comparison of Compressive Strength Result N/mm^2 (28 Days)

5. CONCLUSIONS

Based on the above experimental procedure and test, we conclude as:

1. Use of sugarcane press mud in brick has solved the disposal problem; reduced cost and produced Eco- friendly brick for construction.
2. Reduction of weight of interlocking brick up to 20 % of weight of brick. As compere to normal interlocking bricks the bricks are light weight bricks.
3. In the Compressive strength result observed that block A is Shows moderately effect in increasing strength as compared to conventional brick i.e. greater than 3.5 N/mm^2.

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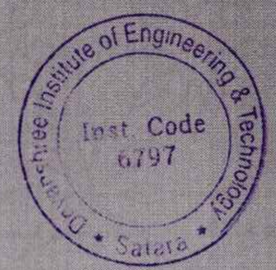

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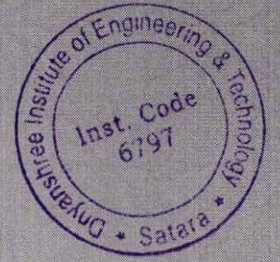

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Article

Effect of Deep Cryogenic Treatment on Corrosion Behavior of AISI H13 Die Steel

Tarang Shinde [1], Catalin Pruncu [2,*], Narendra B. Dhokey [3], Anca C. Parau [4] and Alina Vladescu [4,5,*]

[1] Department of Mechanical Engineering, Finolex Academy of Management and Technology, Ratnagiri 415639, India; tarangshinde@gmail.com
[2] Department of Mechanical Engineering, Imperial College London, Exhibition Rd., London SW7 2AZ, UK
[3] Department of Metallurgy & Materials Science, College of Engineering, Shivajinagar, Pune 411005, India; nbdhokey@yahoo.co.in
[4] Department of Advanced Surface Processing and Analysis by Vacuum Technologies (ReCAST), National Institute of Research and Development for Optoelectronics INOE 2000, 409 Atomistilor St., Magurele, 077125 Bucharest, Romania; anca.parau@inoe.ro
[5] Physical Materials Science and Composite Materials Centre, Research School of Chemistry & Applied Biomedical Sciences, Tomsk Polytechnic University, Lenin's Avenue 30, 634050 Tomsk, Russia
* Correspondence: catalin.pruncu@gmail.com (C.P.); alinava@inoe.ro (A.V.); Tel.: +4021-457-57-59 (A.V.)

Abstract: AISI H13 die steel specimens were subjected to heating at 1020 °C followed by oil quenching and double tempering at 520 °C. Subsequently, these specimens were subjected to deep cryogenic treatment at −185 °C in liquid nitrogen environment for 16 h and then subjected to soft tempering at 100 °C once the specimens attained room temperature. Thereafter, the specimens were subjected to scanning electron microscopy (SEM) analysis and electron backscatter diffraction (EBSD) analysis. The electrochemical corrosion activity was investigated in 3.5% NaCl at 23 ± 0.5 °C by evaluating the evolution of open circuit potential over time and potentiodynamic curves, and electrochemical impedance spectroscopy study was also carried out. The heat-treated specimens exhibited better resistance to corrosion through more electropositive values of open circuit potential. This could be attributed to lower grain boundary area in heat-treated specimens as compared to 16 h cryogenically treated specimen as higher grain boundary areas behave as an anode in an electrochemical cell, thereby enhancing the rate of corrosion. According to electrochemical tests, the cryogenically treated surface is more resistant to corrosion, followed by heated alloy. However, both surface modification treatments improved the corrosion behavior of the untreated alloy.

Keywords: corrosion; deep cryogenic treatment; AISI H13; Nyquist; open circuit potential; tool steels; corrosion rate

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1. Introduction

The tool steels are widely used in hot working industries including forging and extrusion. According to the market study and analysis, the global market size of the tool steels was USD 4.29 billion in 2020, and as per the forecast, their value is expected to cross over USD 6395.95 by 2025 [1,2]. AISI H13 hot work die steel is one of the most important materials belonging to this category. In some of the applications, AISI H13 undergoes a phase change, austenitizing some region of the component when it is under stress and heat due to friction which could promote the spalling [3]; other reasons for failure could be oxidative or abrasive wear, fatigue cracking and chipping as experienced in some of the cases [4]. The corrosion damage of H13 die steel has also been reported in the case of applications involving the manufacturing of dies for aluminum cans [5]. Since these materials are subjected to heat and mechanical stresses, their corrosion behavior needs to be studied in order to investigate their response towards changing conditions, as well as to report any possible degradation in their properties during service.

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The tool steels need to possess certain characteristics in order to withstand highly challenging conditions during mechanical working operations [6]. These characteristics include hardness, resistance to wear and fatigue properties. Even though the basic combinations present in tool steels do add up these characteristics, the need of the time is to further improve them. The deep cryogenic treatment has been recommended as an add-on process to improve certain mechanical properties, especially wear resistance, as indicated by many researchers in their studies on tool steels [7–17]. This treatment increases the density of precipitation of most of the carbides in tool steels and reduces average carbide particle size [18]. Some researchers have elaborated that the deep cryogenic treatment improves the surface finish of the materials through the refinement of martensite laths [19]. This method had been beneficial for materials such as AISI H13 die steel [20–23] in order to improve the wear resistance, hardness and surface characteristics, thereby enhancing the performance of the material.

The fine carbides precipitated after cryogenic treatment assist with the improvement of these properties substantially. The cryogenic treatment even had an impact on fatigue life, as reported by some researchers [24,25]. The fine carbides could act as microcrack arresters. They delay the propagation of these microcracks, thereby enhancing the fatigue life, which is quite beneficial for applications such as mechanical working. It has also been elaborated that the deep cryogenic treatment improved the corrosion resistance for some tool steels such as 1.2080 [26]. Corrosion behavior of hot work tool steels has been studied, and it was reported by researchers that methods such as pressure vapor deposition (PVD) and nitriding could be helpful in improving the corrosion resistance of these materials [27–29]. PVD coatings assist in localizing the attack on the material surface. A good adhesiveness is essential in retaining the coating layer and thereby preventing any further attack of the chemical or any surrounding agent which could result in formation of any corrosive byproduct.

One researcher experimented with the formation of a borided layer on the surface of AISI H13 steel [30]. The borided layers formed single phase or dual phase structures and their layers were compact and crack-free, which improved the corrosion resistance of the steel, especially in the case of steam turbine applications. The deep cryogenic treatment has been beneficial in improving the corrosion characteristics of structural steels. It could certainly become a potential method for those applications where corrosion plays a very important role deciding the lifespan of the component [31]. Most of the treatments have focused upon surface modifications in order to improve the corrosion resistance of tool steels.

In the last few decades, there was an interest in increasing the wear resistance of steels by applying deep cryogenic treatment due to homogenization and stabilization of internal microstructure. The modification of other steels such as D-2, M-2 and AISI H13 by deep cryogenic treatment can be found in the literature. The aim of the paper is to investigate the corrosion resistance of the AISI H13 die steel specimens by the deep cryogenic treatment at −185 °C in liquid nitrogen environment for 16 h and to see the effect of this treatment on its properties. Electron backscatter diffraction (EBSD) analysis, grain mapping tools and Nyquist plots have been used in the current research work in order to evaluate the performance of AISI H13 die steel specimens when subjected to a corrosive atmosphere.

The study also investigates the outcomes based on the experimentation to comment upon the best treatment for H13 steel in order to withstand the corrosive atmosphere. The results have been compared to those of the untreated and conventionally heat-treated specimens.

2. Materials and Methods

2.1. Surface Modification Process

AISI H13 material was procured from Rajasthan Steels, Pune (India), to fabricate the specimens for research work. The chemical composition involves C—0.39%, Mn—0.30%, Si—0.40%, Cr—5.40%, Mo—1.4%, V—1.00%, balance Fe, by weight. A set of AISI H13

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specimens (diameter 8 mm and length 15 mm) was hardened at 1020 °C with a step-by-step heating (first heating to 500 °C at a rate of 7 °C/min and holding for 20 min and thereafter heating to 860 °C and soaking for 20 min) to prevent cracking due to thermal stresses. These specimens were soaked for 20 min at 1020 °C followed by oil quenching. The quenched specimens were double tempered at 520 °C for 2 h. This treatment was named conventional heat treatment for AISI H13 steel and the specimens were designated as "heated" specimens. In a cryo-chamber (Sanmar, Mumbai, India) supplied with liquid nitrogen at a cooling rate of 3 °C/min, the other set of specimens was subjected to conventional heat treatment (step-by-step heating to 1020 °C, oil quenching and double tempering at 520 °C for 2 h) and cryogenic treatment at −185 °C at a cryo soaking period of 16 h [21]. At the end of 16 h of cryo soaking period, these specimens were transferred to an insulated box (Cryobox, Sigma-Aldrich, Irvine, UK) until they attained room temperature, and thereafter the specimens were soft-tempered for 1 h at 100 °C to eliminate the cold stresses produced during the cryogenic treatment. Specimens cryogenically treated for 16 h were referred to as "cryo" specimens.

2.2. Characterization Methods of Developed Surfaces

All specimens were examined by scanning electron microscopy (SEM) (Tabletop Microscope TM3030, Hitachi, Tokyo, Japan) after mechanical polishing which involved grinding down using SiC papers (homemade), sequentially from 320 to 4000 grits. Subsequently, the ground specimens were active oxide polishing (OPS) polished for 30 min using a suspension diluted with H_2O with a ratio of 1:5 to a mirror finish.

The specimens were further characterized for grain orientation and texture direction using a Hitachi 3400 SEM-based Bruker e-flash electron backscatter diffraction (EBSD) detector (Cheshire, UK) at 20 kV acceleration voltage, 10 mA current density and 1 mm step size. The images were postprocessed using MTEX software (free Matlab toolbox, see details in https://mtex-toolbox.github.io/, accessed on 3 December 2021).

The electrochemical behavior was measured using a VERSASTAT potentiostat/galvanostat (Princeton Applied Research, Princeton, NJ, USA). The tests were performed in 3.5% NaCl solution (pH = 7.4) at 23 ± 0.5 °C. Each sample was placed in a Teflon sample holder (Princeton Applied Research, Princeton, USA) with exactly 1 cm^2 exposed to the corrosive media. A platinum electrode (XM140, Radiometer Analytical, Loveland, CO, USA) was used as the counter electrode and saturated calomel (XR110, Radiometer Analytical, Loveland, CO, USA) was used as the reference electrode. All measurements were achieved at a scanning rate of 1 mV/s. The open circuit potential (E_{OC}) was monitored for 1 h, starting right after the immersion of the sample in the 3.5% NaCl solution, and the potentiodynamic curves were recorded at −1 to +2 V vs. E_{OC}. The tests were performed according to the standard ISO 16151:2018. Electrochemical behavior of the investigated specimens was also examined by electrochemical impedance spectroscopy (EIS). Impedance measurements were performed at open circuit potential with constant perturbing AC signal amplitude of 10 mV over a frequency range extending from 0.1 to 10^4 Hz. Analysis of the spectra was performed by equivalent circuit fitting using Zview software (Princeton Applied Research, Princeton, USA). After the corrosion tests, each surface was analysed by SEM and profilometry (Dektak 150, Bruker, Billerica, MA, USA) in order to evaluate the morphology and roughness.

3. Results

3.1. Microstructural Results

The microstructural details of polycrystalline materials can be investigated with the help of electron backscatter diffraction (EBSD) analysis [32]. One of the reasons why grain boundaries must be analyzed is that the performance and integrity of the material depend upon the grain boundary networks present inside the materials [33]. The EBSD feature has made it possible to analyze more than 10^4 boundaries in order to investigate their role in properties such as corrosion resistance [34,35]. The grain size has a significant

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impact on the mechanical and corrosion properties of an alloy. The EBSD phase maps could be useful in identification and distribution of different phases in the alloys. Figure 1 shows the phase maps for untreated, heated and cryo-treated specimens depicting the significant precipitation of different phases in different cases. Figure 2 reports the results obtained through EBSD analysis clarifying that the heat treatment (Figure 2b) and cryogenic treatment (Figure 2c) bring about recrystallized structure in the material. A low angle grain boundary is typically the boundary between two crystal grains with a misorientation of less than 15° [36,37].

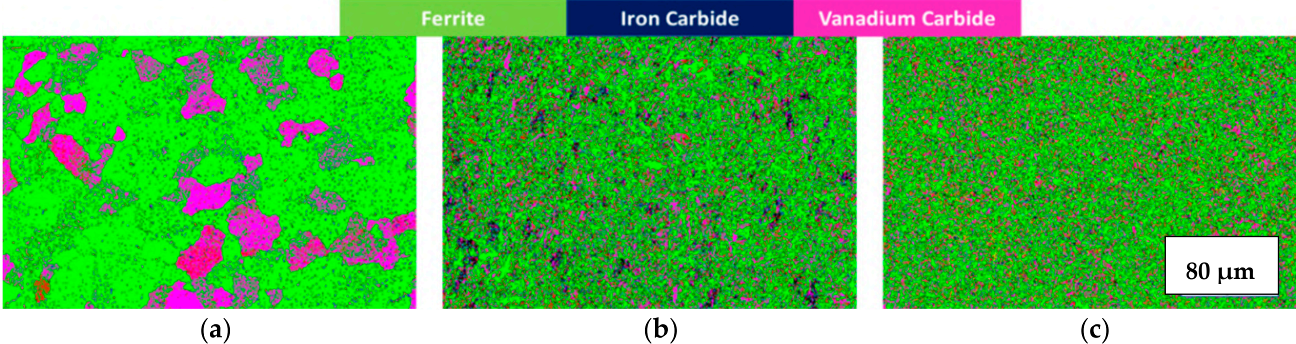

Figure 1. Maps depicting the main phase of studied material: (**a**) untreated, (**b**) heated and (**c**) cryo specimens.

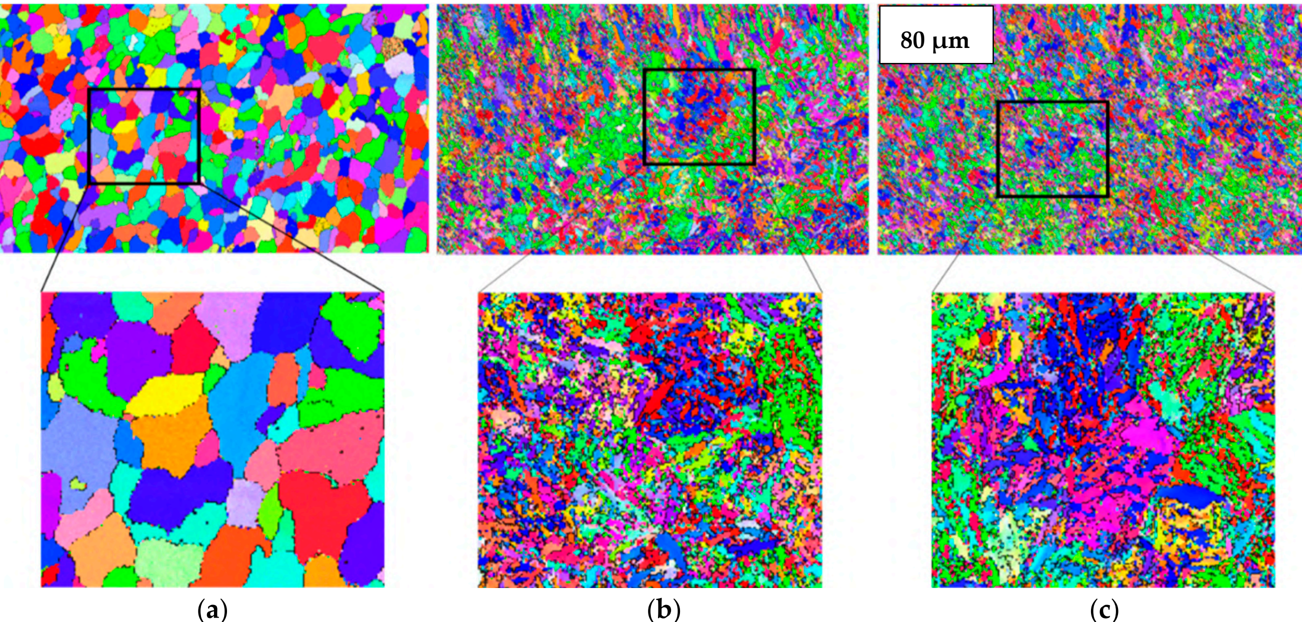

Figure 2. Inverse pole figure map obtained from EBSD analysis for (**a**) untreated, (**b**) heated and (**c**) cryo specimens.

Figure 3 elaborates the low angle grain boundary (LAGB) patterns for untreated, heated and cryo specimens which indicate that the as-received specimen has a limited number of LAGBs (noted by green) while heat-treated and cryo specimens show more LAGBs which could be attributed to the recrystallized structure in both specimens.

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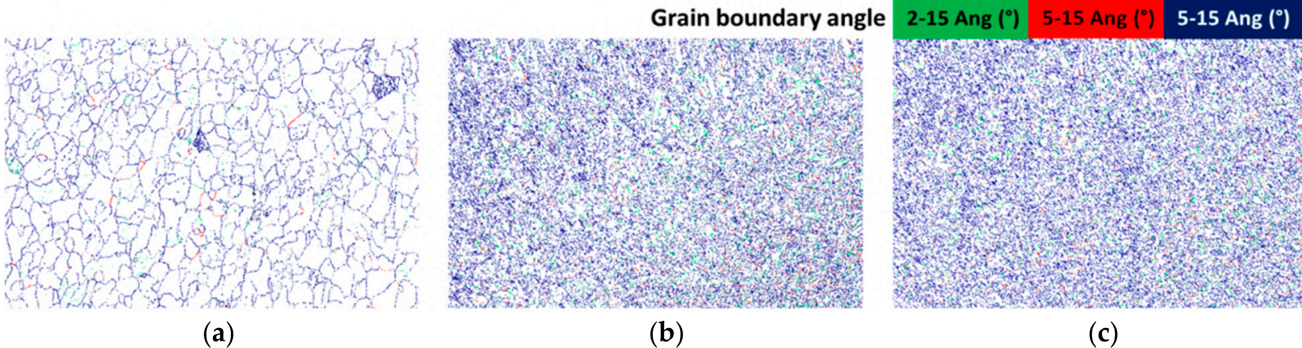

Figure 3. LAGBs for (**a**) untreated, (**b**) heated and (**c**) cryo specimens.

The deep cryogenic treatment of H13 steel resulted in a fine-grained structure. As geometrically necessary dislocation (GND) density measurement by EBSD has become a very popular tool in microstructural analysis [38], Figure 4 presents the geometrically necessary dislocation evolution of untreated, heated and cryo-treated specimens. It is observed from the GND maps that the dislocation density trend is untreated < heated < cryo; the highest dislocation density is found for cryo specimens (10^{14}–10^{15}), which could be attributed to increased plastic strain, resulting in accumulation of dislocations at the grain boundary areas for cryo specimens of H13 steel.

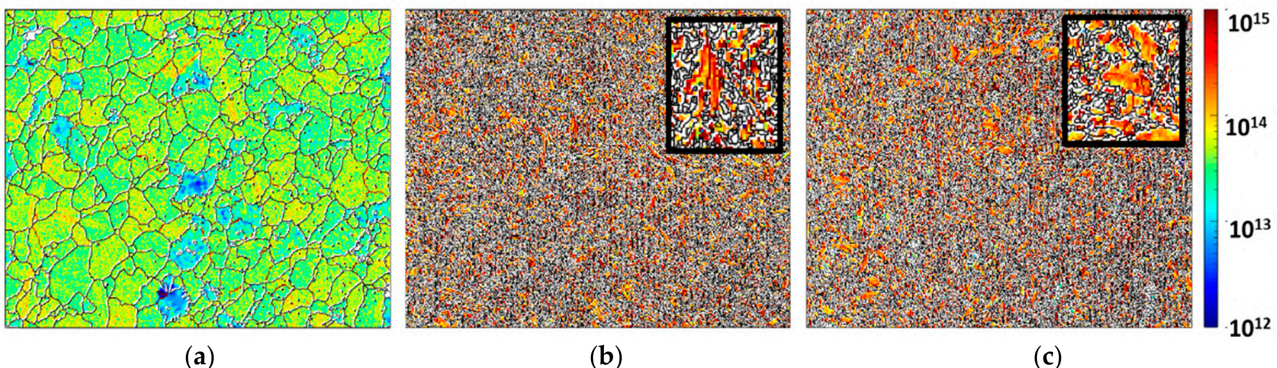

Figure 4. Geometrically necessary dislocation evolution of (**a**) untreated, (**b**) heated and (**c**) cryo-treated specimens.

Figure 5 represents the grain size variation for untreated, conventionally heat-treated (heated) and 16 h cryogenically treated (cryo) specimens. The graph illustrates that the grain size is minimal for 16 h cryogenically treated H13 steel for both conditions (before and after corrosion). The cryogenic treatment assists in the precipitation of fine carbides along the grain boundaries which inhibits the grain growth, resulting in a fine-grained structure [39]. Minimum grain size depicts the maximum grain boundary area inside the material. It has been shown by some authors that the grain refinement leads to increased susceptibility to corrosion [40–42], but the passivity of the film present on the surface plays a vital role in deciding the response of the material to corrosion. When untreated specimens are compared with conventionally treated (heated) ones, there is a reduction in grain size and, as stated by some authors, the corrosion resistance would be greater if the passivity of the film is maintained even for a fine-grained material [43–45].

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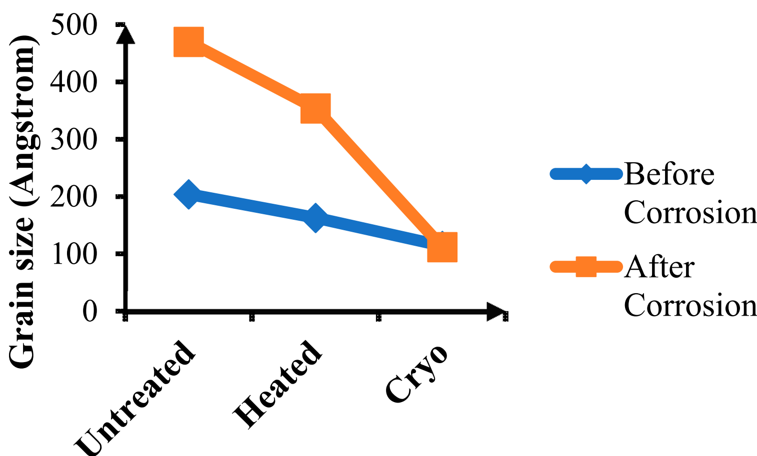

Figure 5. Grain size variation for untreated, conventionally heat-treated and 16 h cryogenically treated specimens before and after corrosion.

3.2. Electrochemical Results

The electrochemical corrosion activity was investigated in 3.5% NaCl at 23 ± 0.5 °C by evaluating the evolution of open circuit potential (OCP) over time (Figure 5) and potentiodynamic curves (Figure 6). The open circuit potential (OCP) is a parameter that is related to the protective ability of the passive film. During 1 h of immersion, the OCP value slightly changed, indicating that steady-state conditions were not reached (Figure 5). Based on the results presented in Figure 6, one may see that the "cryo" specimen has an electropositive value of E_{oc}, indicating a good resistance to NaCl attack.

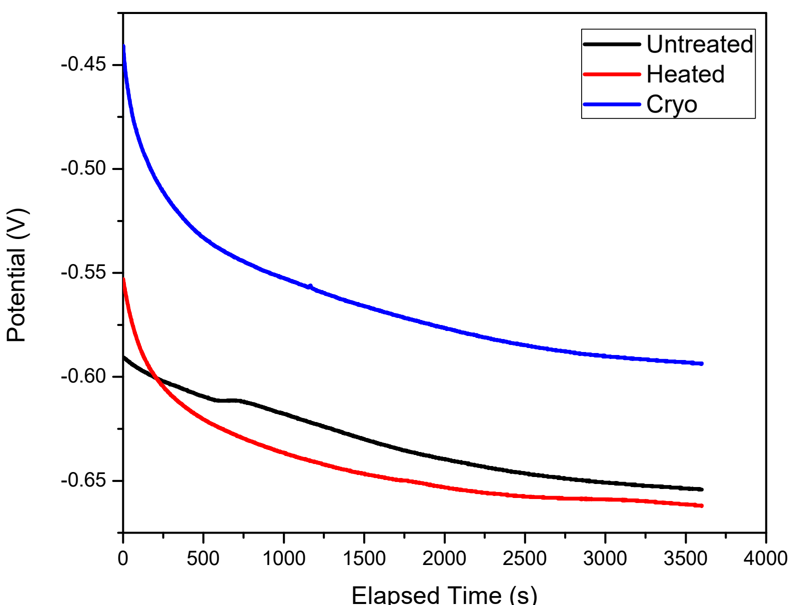

Figure 6. Evolution of the open circuit potential over time.

Figure 7 represents the polarization curves of the investigated surfaces. Based on Tafel extrapolation [46], the main corrosion parameters were extracted from Figure 6 and presented in Table 1, as frequently reported [47]. Heated specimens exhibited a more electropositive value of the corrosion potential (E_i = 0) compared to the uncoated and cryo specimens, indicating that the corrosive solution had less influence on their surfaces. The cryo specimen had a more electronegative corrosion potential, showing poor corrosion resistance to NaCl attack. Lower corrosion current density (i_{corr}) and higher polarization resistance (R_p) values were observed for heated specimens, showing good

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corrosion resistance. Taking into account the electrochemical parameters, it can be said that heated specimens exhibited the best corrosion resistance to 3.5% NaCl, followed by the untreated and the cryo specimens.

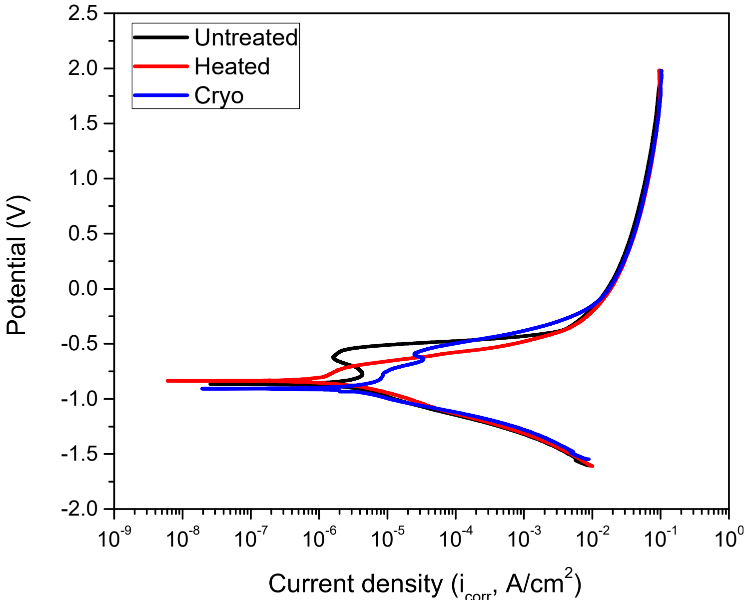

Figure 7. Potentiodynamic curves of investigated surfaces.

Table 1. Main corrosion parameters of untreated, heated and cryo specimens. $E_i = 0$: corrosion potential; i_{corr}: corrosion current density; R_p: polarization resistance; P: porosity; P_e: protective efficiency; CR: corrosion rate.

Sample	$E_i = 0$ (mV)	i_{corr} ($\mu A/cm^2$)	R_p ($k\Omega$)	P	P_e (%)	CR (μm/year)
Untreated	−865	4.387	11.644	-	-	0.051
Heated	−835	1.303	20.917	0.532	70.3	0.015
Cryo	−906	2.005	9.130	1.199	54.3	0.023

The surface porosity (P) was estimated based on Elsner's empirical equation [48,49] taking into account the values of polarization resistance (R_p) before and after applied treatments. Moreover, the protective efficiency (P_e) was also calculated based on the formula reported in [49], considering the ion corrosion densities of the untreated substrate and treated substrates. The corrosion rate (CR) has been estimated according to ASTM G102-89 standard (reapproved 2015) [50], using the following equation:

$$CR = K_i \frac{i_{corr}}{\rho} EW \tag{1}$$

where CR = corrosion rate, $K_i = 3.27 \times 10^{-3}$, ρ = materials density, i_{corr} = corrosion current density and EW = equivalent weight.

Taking into account the values of porosity, one may see that the alloy exhibited low porosity after heating, compared with the cryogenically treated alloy. Moreover, the protective efficiency in response to NaCl attack is higher in the case of the heated alloy when compared to the cryo alloy. The corrosion rate can be estimated in the following order: untreated > cryo > heated alloy. The high corrosion rate of cryogenically treated alloy can be attributed to the high porosity of the surface. However, it can be summarized that the best corrosion resistance was found for the heat-treated alloy and cryogenically treated alloy.

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Electrochemical impedance spectroscopy (EIS) was used to investigate the electrochemical behavior of the investigated systems, highlighting the surface properties. For this purpose, the applied amplitude of the perturbation signal was 10 mV RMS vs. Eoc in a frequency range of 0.1–10^4 Hz. The impedance data were displayed as a Nyquist plot (Figure 8). According to this plot, one may observe that the specimens exhibit a better protection with increasing order from left to right as cryo < untreated < heated.

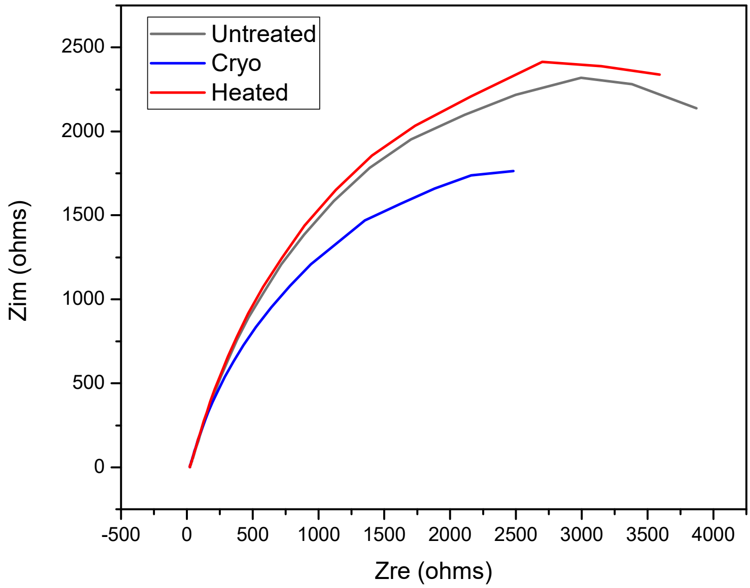

Figure 8. Nyquist plot for the investigated specimens.

3.3. Morphology before and after Electrochemical Tests

The morphology before and after electrochemical tests was evaluated by SEM analysis, and the results are presented in Figure 9. Before electrochemical tests, all surfaces appeared to be without defects or cracks. After corrosion tests, each surface was affected differently. The surface of the untreated alloy was destroyed on all corroded areas. At the magnification of 5000×, some precipitation of oxides of Al, Cr and Fe can be seen, as can the NaCl precipitates from the corrosive solution. The heated alloy is more resistant to corrosion, having a localized corrosion in some parts of the corroded region. On this surface, some oxides of Al, Cr and Fe can be seen, having a high affinity to oxygen. Some holes can also be observed, indicating that the corrosive solution penetrated more deeply. In the case of cryogenically treated alloy, many cracks appeared on the surface after the electrochemical tests. One possibility is that these cracks could be attributed to the destruction of the oxides. The heated AISI H13 steel displays better corrosion resistance compared to untreated AISI H13, and their corrosion resistance is comparable to that of the cryogenically treated alloy. In order to explain more about the corrosion process, the investigation of the roughness of the specimens was performed before and after corrosion tests.

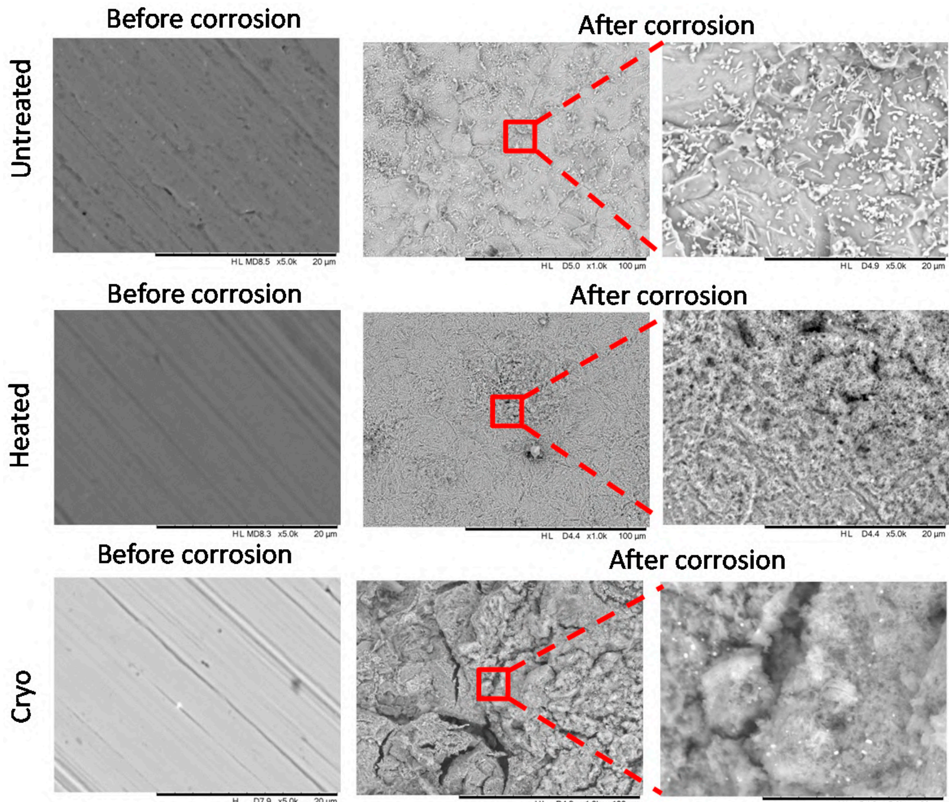

Figure 9. SEM images of the investigated specimens before and after corrosion.

3.4. Roughness before and after Electrochemical Tests

The roughness was evaluated on a length of 10 mm by a surface profilometer (Dektak 150, Bruker, Billerica, MA, USA) equipped with a 2.5 µm diameter stylus. For evaluation of roughness, two main parameters were calculated: arithmetic average deviation from the mean line (R_a) and skewness (S_{kew}). In Figure 10, the histograms of both calculated roughness parameters captured before and after electrochemical tests are presented. According to the R_a parameter, one may note that the roughness of all investigated surfaces was significantly increased after corrosion tests, indicating that all surfaces were affected by the corrosive solution. The heated and cryo alloys exhibited almost similar values of R_a, indicating that both surfaces exhibited comparable corrosion behavior. More relevant is the skewness parameter. A negative value of S_{kew} implies a surface formed of many valleys, while a positive value suggests a surface formed of mainly peaks and asperities. A S_{kew} with a value close to 0 suggests a flat surface. Taking into consideration these states, one may see that the untreated surface has an almost flat surface before corrosion and a surface with many peaks after corrosion. This finding demonstrated that the corrosive solution digs into the material and forms many pits. The heated alloy has a negative S_{kew} before corrosion tests, meaning that the surface has many valleys, which are transformed to high peaks after corrosion tests, indicating that the surface is significantly affected by the corrosive solution. The same phenomenon was also found in the case of the cryogenically treated surface. Note that the differences found for the cryo surface are small compared to those found for the heated surface, indicating that the cryo surface is more resistant to corrosion in 3.5% NaCl.

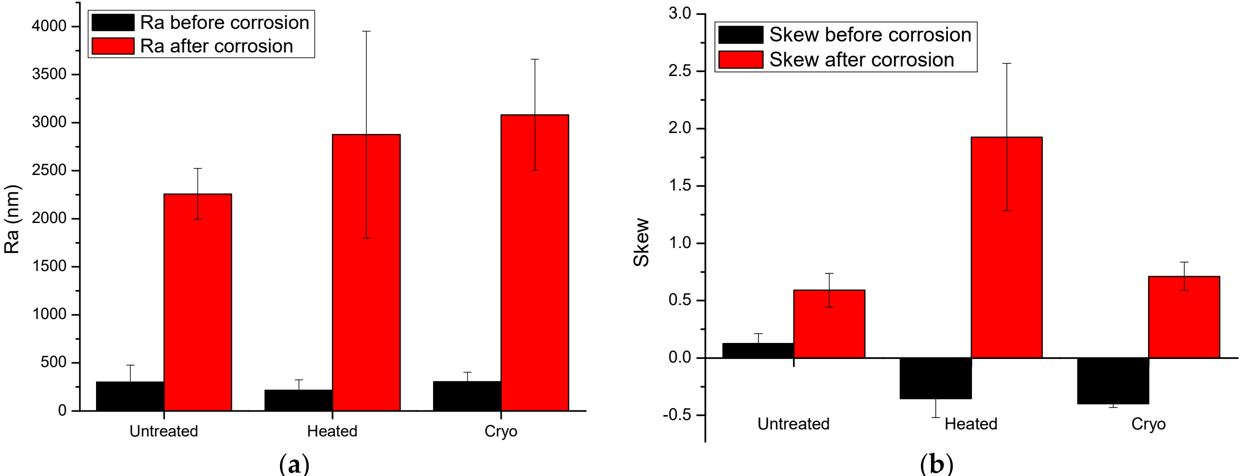

Figure 10. Roughness parameters: (**a**) R_a—arithmetic average deviation from the mean line; (**b**) S_{kew}—skewness of the investigated specimens before and after corrosion resistance.

4. Discussion

Based on the results obtained through corrosion tests, it can be said that the grain boundaries play a very important role in deciding the properties of materials under different service conditions for all types of treatments. High geometrically necessary dislocation (GND) density has been noted for 16 h cryogenically treated AISI H13 specimens (cryo specimens), which could be attributed to the increased plastic strain or strain hardening resulting in the accumulation of dislocations at the grain boundary areas. A reduction in grain size has been observed in the case of 16 h cryogenically treated specimens (cryo specimens), which could be attributed to the precipitation of fine carbides at the grain boundaries inhibiting the grain growth [12]. The highest values of open circuit potential (OCP) for AISI H13 cryo specimens indicate that these specimens are good in resisting the attack of NaCl as compared to untreated and heated specimens. The heated specimens have shown good results in the case of polarization curves with Tafel's extrapolation and estimation of surface porosity as compared to heated and cryo specimens. This indicates that the heated specimens form a protective and passive layer on the surface of the specimen. The Nyquist plots are also in agreement with the results for heated specimens. The SEM plots indicate a broken film in the case of cryo specimens, which could have resulted in increased corrosion; however, the reasons for the breakage of the film need to be determined.

Surface roughness evaluations are essential in analysing the response of materials to corrosive environments. There was a significant increase in the roughness for all types of specimens as an effect of the corrosive atmosphere. The skewness parameters evaluated for untreated, heated and cryo specimens indicate that the cryo specimens have better corrosion resistance as compared to the other two types, which could be attributed to the less significant impact of the corrosive atmosphere on cryo specimens.

One of the important aspects that need to be discussed is the influence of mechanical properties on the corrosion behavior of the materials. In this case, if the hardness is compared amongst the three categories (i.e., untreated, heated and cryo-treated), it can be seen that the hardness increases as follows: untreated (42 HRC) < heated (50 HRC) < cryo (56 HRC). The increase in hardness could be the result of the conversion of retained austenite into martensite after cryogenic treatment and the precipitation of fine carbides in the matrix of tempered martensite [12,21]. The grain refinement is also one of the reasons for the improvement in hardness. Based on these results for hardness, it can be stated that increased martensitic content indicates better corrosion resistance as in the case of cryo specimens [51].

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In summary, heated and cryo specimens of AISI H13 exhibit better resistance to corrosion as compared to untreated material, and deep cryogenic treatment is useful in improving the corrosion resistance.

5. Conclusions

The current research work was aimed at investigating the corrosion behavior of cryogenically treated AISI H13 die steel. The following points summarize the major outcomes:

- The deep cryogenic treatment is beneficial in improving the corrosion resistance of AISI H13 material and could be useful in applications involving high mechanical stress and a corrosive environment.
- The grain boundaries decide the corrosion characteristics of the material; increased geometrically necessary dislocations indicate higher plastic straining. There is a reduction in grain size for AISI H13 specimens subjected to 16 h deep cryogenic treatment, which is attributed to the precipitation of fine carbides at the grain boundary areas.
- Parameters such as higher open circuit potential and skewness indicate that H13 specimens subjected to deep cryogenic treatment have a better response to a corrosive environment, whereas the surface porosity measurements and Nyquist plots confirm the superior response of heated specimens to a corrosive atmosphere.

Overall, it can be concluded that deep cryogenic treatment is useful in improving the corrosion properties of H13 die steel. Some further investigations of material surfaces exposed to a high-temperature corrosive environment could also reveal some important outcomes for H13 steel.

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Selection of optimum plant layout using AHP-TOPSIS and WASPAS approaches coupled with Entropy method

Anand S. Shivade[a] and Sagar U. Sapkal[a*]

[a]*Department of Mechanical Engineering, Walchand College of Engineering, Sangli. 416415, Shivaji University, Vidyanagar, Kolhapur. 416 004 (MH), India*

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ABSTRACT

Layout design and selection often have notable effects on the performance of the manufacturing industry. This research investigates the Multi-Criteria Decision Making (MCDM) approach to find out the optimum plant layout design. The proposed methodology is demonstrated through the real-life setting for the gearbox manufacturing industry. Manual and computerized layout generation approach is used efficiently and accordingly, six layout designs are generated. The approach takes into account qualitative as well as quantitative performance criteria for the selection of layout design. Analytical Hierarchy Process (AHP) is applied to obtain the weight of qualitative measures. Ranking of alternatives is obtained through the application of Technique for Order Preference by Similarity to Ideal Solution (TOPSIS) and Weighted Aggregated Sum-Product Assessment (WASPAS) both integrated with the Entropy method. Empirical findings indicate that the rank acquired using the TOPSIS method is perfectly parallel to those acquired through the WASPAS method, which confirms the applicability and potential of these methods. Also, the effect of the parameter λ in WASPAS method on performance score is stable. At the same time, this paper analyses the rank reversal phenomenon and proves that the ranking proposed by TOPSIS satisfies ranking stability.

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1. Introduction

Plant layout is an arrangement of sections, subsections, departments, stations and storage space within the existing or proposed facility. The effective layout of the industry is of enormous significance for its effective use. Therefore to meet the required effectiveness, setup should be able to quickly shift gears from one product to another without major alterations regarding available resources. Manufacturing industries producing the standard product such as gearbox require a lot of variation in the product and at the same time should be able to fulfil the growing demands in the market. The layout is classified as static, dynamic, multi-objective, multi-floor, equal area and unequal area. Among these, the design and selection of unequal area facility layout problems are the most critical task. This problem considers a set of rectangular areas and rectangular sub-areas (manufacturing facilities) and should be positioned so that they do not overlap in the production region. The selection of appropriate layout design is a Multi-Criteria Decision Making (MCDM) approach. This approach requires consideration of the qualitative and quantitative criteria, jointly. Qualitative measures include layout flexibility, maintenance, accessibility, human issue, plant safety and information flow while quantitative measures include layout cost or material handling cost, adjacency score i.e. closeness request, distance score, aspect ratio and production volume (Aiello et al., 2012; Yang & Kuo, 2003). A proper plant layout can improve efficiency and reduce material handling costs. Singh & Sharma (2006) focused on current and future research trends on layout problems of formulations and solution

* Corresponding author.
E-mail address: sagarus1201@gmail.com (S. U. Sapkal)

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methodologies. Pérez-Gosende et al. (2021) reviewed literature and performed an analysis based on manufacturing facility characteristics by configuring layout types, layout planning steps, material handling systems, and generation and evaluation of alternatives. Systematic Layout Planning (SLP) is a procedural approach and is successfully applied to solve real life problems of manufacturing industries to improve plant layout (Naqvi et al., 2016; Wiyaratn & Watanapa, 2010).

The construction algorithmic approach such as Automated Layout Design Program (ALDEP), Computerised Relationship Layout Planning (CORELAP) and BLOCPLAN has been proposed to create layout design alternatives (Hakim & Istiyantri, 2015; Rajesh et al., 2016; Tambunan et al., 2018). Hari Prasad et al. (2014) tackled the existing layout situation using the Computerized Relative Allocation of facilities Technique (CRAFT) approach for layout cost optimization. In addition commercial software like spiral and algorithms, Plant Layout Analysis and Evaluation Technique (PLANET), Computerised Facility Design (COFAD) were developed to resolve single floor layout problem whereas for multi floor design, Multi-floor Plant Layout Evaluation (MULTIPLE), Micro CRAFT (MCRAFT), Layout Optimization with Guillotine Induced Cuts (LOGIC) have been introduced by Hadi-vencheh & Mohamadghasemi (2013) and Moatari-Kazerouni et al. (2015).

Abdul-Hamid et al. (1999) presented AHP approach to select an appropriate layout design by considering three objectives viz. flexibility, production volume and manufacturing costs. Yang & Kuo (2003) in their YK model, proposed AHP and Data Envelopment Analysis (DEA) approach for ranking layout design. Also, Ertay et al. (2006) presented a similar methodology to rank the facility layout design. Case studies on railway system improvement and optimization using AHP/DEA methodology with computer simulation are reported by Azadeh et al. (2008). Yang & Hung (2007) applied the TOPSIS & fuzzy TOPSIS approach for layout design ranking and compared their results with the YK model. Sharma & Singhal (2017) applied fuzzy TOPSIS for selection of the best procedural approach of layout design. Agarwal & Singholi (2018) analysed AHP-TOPSIS and Fuzzy AHP- Fuzzy TOPSIS approach for alternative layout designs and compared with the existing design. Yang et al. (2013) used rough set theory, AHP and TOPSIS to choose the best energy efficient layout design alternative among the proposed layouts. Hadi-vencheh & Mohamadghasemi (2013) proposed NonLinear Programming model (NLP) in correlation with AHP to solve the facility layout problem. Kong (2011) studied the causes of rank reversal in TOPSIS and proposed subjective preferences set by decision-makers that helps for more scientific decision-making. Aires & Ferreira (2019) defined a framework to evaluate the TOPSIS method and suggested models related to the different cases of rank reversal. García-Cascales & Lamata (2012) studied the causes of rank reversal in the TOPSIS related to the normalization method. There is a scope for applying improved methods such as AHP-TOPSIS and WASPAS by integrating with the Entropy method for selection of optimum layout design for the problem under consideration i.e. modification of plant layout of the gearbox manufacturing industry. It is worth mentioning that no literature reported on rank reversal related to layout selection. The present study is focused on finding the optimum layout design for the unequal area and irregular shape department of a gearbox manufacturing plant by considering both qualitative and quantitative measures. Section 1 consists of an introduction and literature survey. Section 2 includes data collection and analysis of existing layout. Section 3 articulates generation of alternative layouts. In Section 4, the details of performance measures and application of the AHP approach are presented. Section 5 analyses two MCDM approaches for optimum layout selection. Section 6 proposes a rank reversal study. Section 7 highlights the conclusion related to this study.

2. Data collection and existing plant layout

The industry manufactures standard as well as custom range planetary gearbox of various models. The list of various departments and the area requirement for each department is summarized in Table 1. A block layout of the existing plant layout of these 14 departments is as shown in Fig. 1. The industry is facing a shortage of supply of products that lead to not satisfying the demands and thus the scope is identified in terms of modification and optimization of the layout. It has been analysed that there is a necessity for improving the existing plant layout by applying systematic approaches that will lead to improving productivity with effective utilization of resources. Various alternatives have been studied for comparison and for suggesting the optimum solution.

Table 1
Departments and their area

Sr. No.	Name of Department	Area
0	Office	660.00
1	Raw material store-A	2625.00
2	Raw material store-B	450.00
3	Fabrication shop	392.00
4	Machine shop	9646.00
5	Quality control	1802.00
6	Store	3131.25
7	Cleaning and lapping	148.50
8	Assembly shop	1772.25
9	Test Running	1002.00
10	Painting shop	420.75
11	Packing shop	198.00
12	Dispatch	357.00

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13	Maintenance	470.25

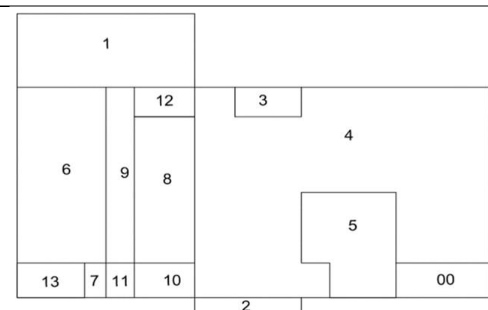

Fig. 1. Existing plant layout

3. Generation of alternative layout designs

3.1 SLP approach

By using the SLP approach the layout is generated as mentioned below. For different sections/ activities of production of a single gearbox, From-To-Chart as shown in the Table 2 is constructed which represents the interaction between departments and rectilinear distance. By the addition of these, flow distance in the existing situation is 960.11 feet.

Table 2
From-To-Chart for existing layout

From/To	1	2	3	4	5	6	7	8	9	10	11	12	13
1.Raw material store-A	0	0	0	159.68	0	0	0	0	0	0	0	0	0
2.Raw material store-B	0	0	0	88.04	0	0	0	0	0	0	0	0	0
3.Fabrication shop	0	0	0	66.68	0	0	0	0	0	0	0	0	0
4.Machine shop	0	0	0	0	35.01	0	0	0	0	0	0	0	0
5.Quality control	0	0	0	35.01	0	154.19	0	0	0	0	0	0	0
6.Store	0	0	0	0	0	0	64.25	50.5	0	0	0	0	0
7.Cleaning and lapping	0	0	0	0	0	64.25	0	0	0	0	0	0	0
8.Assembly shop	0	0	0	0	0	0	0	0	25.75	0	0	0	0
9.Test Running	0	0	0	0	0	0	0	25.75	0	68.75	0	0	0
10.Painting shop	0	0	0	0	0	0	0	0	0	0	18.75	0	0
11.Packing shop	0	0	0	0	0	0	0	0	0	0	0	103.5	0
12.Dispatch	0	0	0	0	0	0	0	0	0	0	0	0	0
13.Maintenance	0	0	0	0	0	0	0	0	0	0	0	0	0

In the present study, for identifying the relative importance between the departments and for selecting the reasons of the closeness for the departments, the opinions of industry experts are taken into account. Table 3 summarizes the different reasons for closeness of the departments.

Table 3
Reasons for closeness

Sr. No.	Reason
1	Material flow
2	Supervision and control
3	Share the same personnel
4	Communication need

To decide the ranking of the relationship between departments, the ranking system mentioned by Muther & Hales (2015) is used and is reported in Table 4.

Table 4
Closeness values and their rating of the relationship chart

Value	Closeness	Rating	Colour Code	Meaning
A	Absolutely necessary	6		Must be next to each other
E	Especially important	5		Need to be very close
I	Important	4		Need to be on same floor, side or wing
O	Ordinary closeness Okay	3		Occasional interaction.
U	Unimportant	2		Infrequent interaction.

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X	Not Desirable	1		Keep separate and away

Based on the reasons for closeness, the relationship chart is presented in Fig. 2.

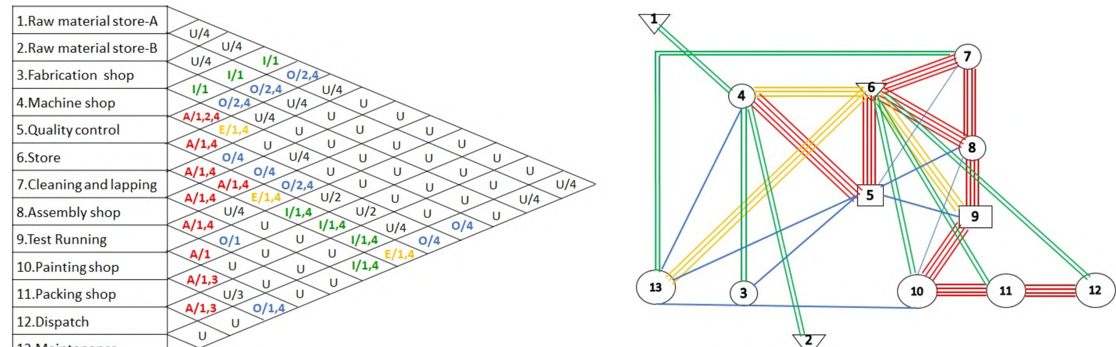

Fig. 2. Relationship chart **Fig. 3.** Activity relationship diagram

Accordingly, activity relationship diagram (Fig. 3) and space relationship diagram (Fig. 4), are developed.

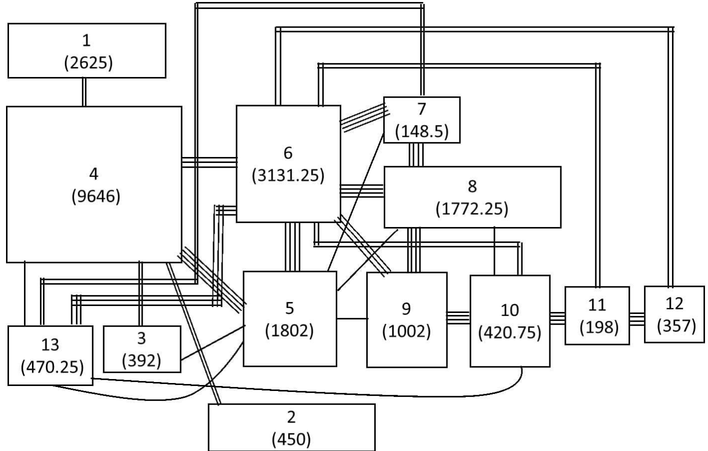

Fig. 4. Space Relationship Diagram

For designing the layout based on SLP method, the following requirements are considered.

1. Location of office, raw material store A and raw material store B are kept as it is.
2. Location of quality control and maintenance department is kept at the outer side of layout for ease of loading and unloading of semi-finished material came from vendors and gearboxes came for maintenance purpose. After applying all conditions, layout alternative is developed (Fig. 5).

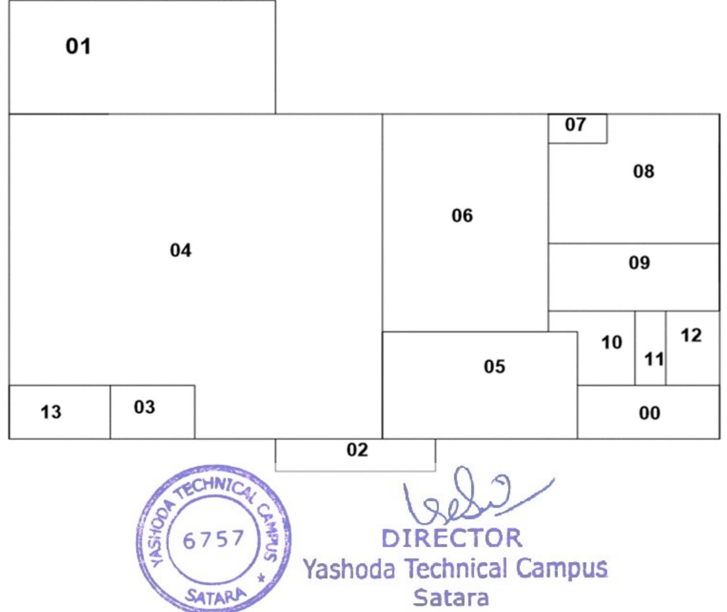

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Fig. 5. Layout based on SLP method

3.2 Flow patterns

Based on the flow patterns, we generate two layout designs as S flow pattern (Fig. 6) and U flow pattern (Fig. 7). In these designs, the arrangement of departments is made as per the operations sequence and production tasks.

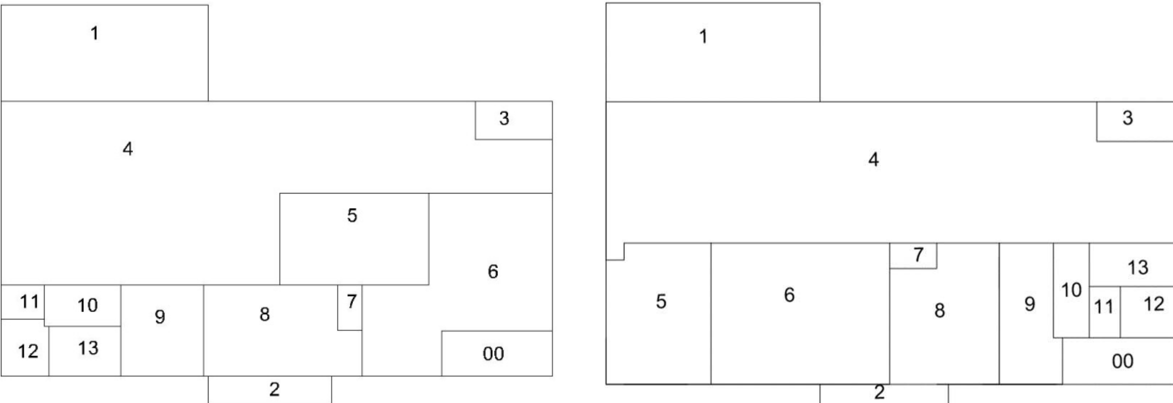

Fig. 6. Layout based on "S" shaped flow pattern **Fig. 7.** Layout based on "U" shaped flow pattern

3.3 ALDEP algorithm

This approach constructs the design by putting the departments in the layout successively. A layout score is calculated after putting all the departments. Table 5 shows the symbols and values mentioned by Panneerselvam (2017) to indicate the degree of closeness between departments.

Table 5
Symbols and values of Closeness

Closeness	Notations	Value
Absolutely necessary	A	64
Especially important	E	16
Important	I	4
Ordinary closeness Okay	O	1
Unimportant	U	0
Not Desirable	X	-1064

In the layout matrix, placement of department is from upper left corner to lower right corner whereas sweep width is user defined. In the present study, considering sweep width 5, minimum department preference is A, E and I, layout matrix size is 55 × 40 and 1 Cell equals to 9.09 Square Feet. Table 6 indicates the number of the squares for all the departments.

Table 6
Department and number of squares

Department No.	0	1	2	3	4	5	6	7	8	9	10	11	12	13
No. of Square	73	289	50	43	1061	198	345	16	195	110	46	22	39	52

In the first design, layout generation starts from a random selection of departments (department No.3). Department No. 4 has an "A" relationship with department No. 3, therefore its placement is next to department 3. Similarly, departments 5,6,7,8,9,10,11 and 12 has "A" relationship with departments 4,5,6,7,8,9,10 and 11, respectively, so they are placed next to previously placed departments. In the placement sequence, only department 13 is left which is placed at last and accordingly layout is generated as shown in Fig. 8. Applying the same procedure, ten alternative layouts are generated. After the generation of alternative layout designs, layout score is computed. Table 7 displays the closeness rating between the departments and the layout score of the first alternative. Similarly, for the remaining ten layout designs, the computed score is listed in Table 8.

Table 7
Closeness of department and layout score

Department	3-4	4-5	4-6	5-6	6-10	6-7	6-8	7-8	7-9	8-9	9-10	9-11	9-12	9-13	10-11	11-12	12-13	6-9	Layout score
Closeness	I	A	E	A	I	A	A	A	U	A	A	U	U	U	A	A	U	E	

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Value	4	64	16	64	4	64	64	64	0	64	64	0	0	0	64	64	0	16	**616**

Fig. 8. Layout generated by ALDEP

Table 8
Alternative layout score and respective rank

Alternative Layout	Sequence	Score	Rank
1	3-4-5-6-7-8-9-10-11-12-13	616	1
2	4-5-6-7-8-9-10-11-12-13-3	594	3
3	5-4-6-7-8-9-10-11-12-13-3	530	8
4	6-5-4-3-7-8-9-10-11-12-13	468	9
5	7-8-9-10-11-12-6-5-4-3-13	562	4
6	8-6-7-13-9-10-11-12-5-4-3	438	10
7	9-10-11-12-6-7-8-5-4-3-13	546	6
8	10-11-12-6-7-8-9-5-4-3-13	547	5
9	11-12-6-5-4-3-13-7-8-9-10	418	11
10	12-11-10-9-8-7-6-5-4-3-13	616	2
11	13-6-7-8-9-10-11-12-5-4-3	535	7

The alternative of having a maximum score is to be considered as the solution. Table 7 shows the maximum score as 616, but it is for two alternatives 1 and 10. To resolve this tie for selection of the first rank, total flow distance and practical limitations are considered and based on that alternative is ranked as 1.

3.4 CRAFT algorithm

The first alternative (Fig. 8) developed from ALDEP is considered as the existing layout (starting point). Possible pairwise interchanges of the departments are taken into account. For all these pairs, assuming 1 trip per hour and ₹1 per trip, layout cost is calculated using Eq. (1) given by Deshpande et al. (2016).

$$\min z = \sum_{i=1}^{m}\sum_{j=1}^{m} f_{ij}c_{ij}d_{ij} \tag{1}$$

where,

z is layout cost per hour
m is number of the department
f_{ij} is the number of trips between departments i and j
c_{ij} is the cost to make one trip between departments i and j
d_{ij} is the distance from department i to j

After carrying pairwise interchange for possible departments, the interchange of departments 6 and 7 gives the minimum cost of ₹ 739.07. This cost is less than the cost ₹ 960.11 of the existing layout. This change is accepted and accordingly the interchange of the selected pair of the departments is done. In the second iteration, possible interchanges are identified and the computed costs for each interchange are summarized in Table 9.

Table 9
Pairwise interchange and respective cost

Pair No.	Pairwise interchange	Cost per hour	Pair No.	Pairwise interchange	Cost per hour	Pair No.	Pairwise interchange	Cost per hour
1	3-4	749.26	7	7-6	739.07	13	9-11	883.16
2	4-5	855.44	8	6-10	1024.28	14	9-12	875.06
3	4-7	1113.15	9	6-9	940.30	15	9-13	1024.92
4	4-6	930.21	10	6-8	858.96	16	10-11	802.99
5	5-6	845.95	11	8-9	770.19	17	11-12	797.97
6	5-7	768.83	12	9-10	871.19			

From Table 9 it is clear that the costs pair-wise interchange is least for pair No.7 and therefore, this is considered as stopping criteria for the algorithm. The solution in term of layout from the CRAFT algorithm is shown in Fig. 9.

Fig. 9. Layout generated by CRAFT

3.5 CORELAP method

Using the relationship chart, Total Closeness Rating (TCR) is computed for all the departments. Relationship between departments in the existing layout is shown in Table 10 while TCR and rank of each department are shown in Table 11.

Table 10
Relationship of department

Department	3	4	5	6	7	8	9	10	11	12	13
3.Fabrication shop	-	I/1	O/2,4	U/4	U	U	U	U	U	U	U
4.Machine shop	I/1	-	A/1,2,4	E/1,4	U	U/4	U	U	U	U	O/4
5.Quality control	O/2,4	A/1,2,4	-	A/1,4	O/4	O/4	O/2,4	U/2	U/2	U/4	O/4
6.Store	U/4	E/1,4	A/1,4	-	A/1,4	A/1,4	E/1,4	I/1,4	I/1,4	I/1,4	E/1,4
7.Cleaning and lapping	U	U	O/4	A/1,4	-	A/1,4	U/4	U	U	U	I/1,4
8.Assembly shop	U	U/4	O/4	A/1,4	A/1,4	-	A/1,4	O/1	U	U	U
9.Test Running	U	U	O/2,4	E/1,4	U/4	A/1,4	-	A/1	U	U	U
10.Painting shop	U	U	U/2	I/1	U	O/1	A/1	-	A/1,3	U/3	O/1,4
11.Packing shop	U	U	U/2	I/1	U	U	U	A/1,3	-	A/1,3	U
12.Dispatch	U	U	U/4	I/1	U	U	U	U/3	A/1,3	-	U
13.Maintenance	U	O/4	O/4	E/1,4	I/1,4	U	U	O/1,4	U	U	-

Table 11
TCR values and placement sequence

Department	3	4	5	6	7	8	9	10	11	12	13	TCR	Placement Sequence
3.Fabrication shop	0	4	3	2	2	2	2	2	2	2	2	23	11
4.Machine shop	4	0	6	5	2	2	2	2	2	2	3	30	7
5.Quality control	3	6	0	6	3	3	3	2	2	2	3	33	3
6.Store	2	5	6	0	6	6	5	4	4	4	5	47	1
7.Cleaning and lapping	2	2	3	6	0	6	2	2	2	2	4	31	6
8.Assembly shop	2	2	3	6	6	0	6	3	2	2	2	34	2
9.Test Running	2	2	3	5	2	6	0	6	2	2	2	32	4
10.Painting shop	2	2	2	4	2	3	6	0	6	2	3	32	5
11.Packing shop	2	2	2	4	2	2	2	6	0	6	2	30	8
12.Dispatch	2	2	2	4	2	2	2	2	6	0	2	26	10
13.Maintenance	2	3	3	5	4	2	2	3	2	2	0	28	9

As an illustration, the department No.6 (store) has the largest TCR, so it is selected at first place in placement order. In second place, the department No.5 having the strongest relationship with the department No.6 is placed. This is repeated for placing the remaining departments in placement sequence and accordingly placement order is generated as 6-5-8-7-4-9-13-10-11-12-3. As per placement rule of CORELAP, department No.6 (first in the sequence) is placed at the center of layout. The department No.5 (second in the sequence) is kept adjacent to department No.6. For placing department No.8 which is at third place in sequence, three combinations are possible. Therefore, based on maximum Placement Rating (PR) the position of department No. 8 in the sequence is decided. PR is expressed in terms of the sum of the weighted closeness rating and their values are mentioned in Table 12 as taken from Panneerselvam (2017).

Table 12
Weighted closeness rating values

Closeness relationship	Pre-assigned	A	E	I	O	U	X
Weighted Rating	729	243	81	27	9	1	-729

PR calculation in the present study is illustrated as follows. In the first arrangement, department No. 8 is placed adjacent to department No. 6 then the closeness relationship between them is “A” having weighted rating 243. Similarly, in the second arrangement department No. 8 is placed adjacent to departments No. 6 and department No. 5, hence, the close relationship between them is “A” and “O” while weighted rating is 243 and 9 which gives PR equal to 243 + 9 = 252. As per the third arrangement, department No. 8 can be placed adjacent to department No. 5, then the closeness relationship between them is “O” whereas weighted rating is 9. Based on maximum PR the second arrangement is considered. These steps of identifying the closeness and calculating the placement rating are repeated for the placement of remaining departments and accordingly a new layout as shown in the Fig. 10 is generated.

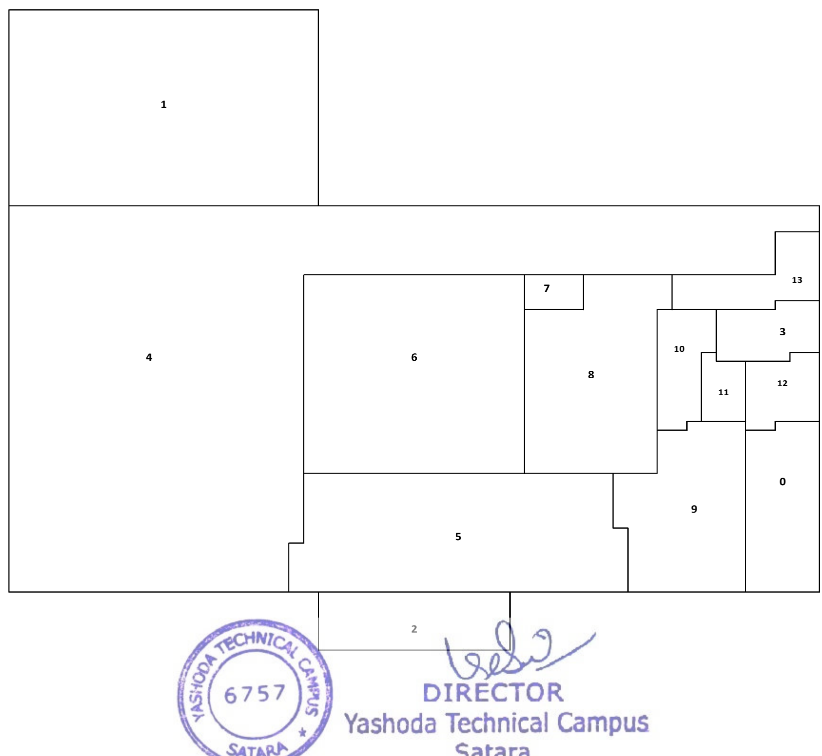

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Fig. 10. Layout generated by CORELAP

4. Performance measures and AHP method

To evaluate the performance of the generated layouts, various metrics such as flow distance, adjacency score, aspect ratio, production volume, cost of the manufactured component, flexibility, accessibility, maintenance, human issue, information flow, machine reconfigurability, and quality factors are available in the literature (Abdul-Hamid et al., 1999; Agarwal & Singholi, 2018; Aiello et al., 2012; Goyal et al., 2012). Based on expert advice from the industry and based on literature study, the qualitative performance measures selected are flexibility, accessibility, maintenance, and human issue and quantitative performance measures considered are flow distance and adjacency score. The objectives are to minimize flow distance and to maximize remaining five performance measures. For finding the value of the qualitative measures, AHP method which has certain advantages as mentioned by Ertay et al. (2006) is applied. The decision hierarchy of AHP in this case is shown in Fig. 11. The terms A1, A2, A3, A4, A5, and A6 are used for layout generated by SLP, "S" type flow, "U" type flow, ALDEP, CRAFT and CORELAP, respectively.

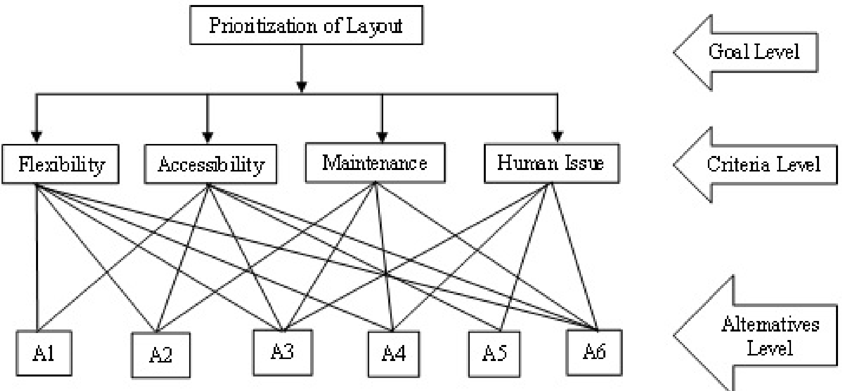

Fig. 11. Decision hierarchy for qualitative criteria in the layout selection

In the layout design problem, all the performance measures will not have the same importance. Therefore, a pairwise comparison is applied in the second step to determine the comparative significance of the performance and alternatives (layouts) according to their influences. Construction of a comparative matrix by a pairwise comparison based on designer preference can be done using a numerical scale shown in Table 13 as mentioned in Yang & Kuo, (2003).

Table 13
Nine-point intensity of importance scale

Intensity of Importance	Definition
1	Equal Importance
3	Moderate Importance
5	Strong importance
7	Very strong importance
9	Extreme importance
2,4,6,8	Intermediate values between the two adjacent judgments

The comparison matrices for all the four criteria are generated using numerical scale values. The matrix for flexibility criteria is shown in Table 14 as an example.

Table 14
Pairwise comparison matrix of flexibility

Alternative	A1	A2	A3	A4	A5	A6
A1	1	3	3	7	7	7
A2	1/3	1	3	5	5	5
A3	1/3	1/3	1	5	5	3
A4	1/7	1/5	1/5	1	1/3	1/3
A5	1/7	1/5	1/5	3	1	2
A6	1/7	1/5	1/3	3	1/2	1

After developing the comparison matrix, normalization is done. For example, in the case of alternative 1, the normalised value is calculated as 1/ (1+ 0.33 + 0.33 + 0.14 + 0.14 + 0.14) = 0.477. Subsequently arithmetic mean is computed that gives criteria weight. For alternative 1 criteria weight equal to (0.477 + 0.608 + 0.388 + 0.292 + 0.372 + 0.382) / 6 = 0.420. Table 15 displays a normalized matrix and criteria weight for flexibility criteria.

Table 15
Normalised matrix and criteria weight

Alternative	A1	A2	A3	A4	A5	A6	Criteria weight
A1	0.477	0.608	0.388	0.292	0.372	0.382	0.420
A2	0.159	0.203	0.388	0.208	0.265	0.273	0.249
A3	0.159	0.068	0.129	0.208	0.265	0.164	0.166
A4	0.068	0.041	0.026	0.042	0.018	0.018	0.035
A5	0.068	0.041	0.026	0.125	0.053	0.109	0.070
A6	0.068	0.041	0.043	0.125	0.027	0.055	0.060

Then, weighted sum is the sum of product of each value in a pairwise comparison matrix to criteria weight of the corresponding alternative. For example, in case of alternative 1, the weighted sum equals (0.420 ×1) + (0.249 ×3) + (0.166 × 3) + (0.035 ×7) + (0.070 ×7) + (0.060 ×7) = 2.82. This weighted sum is divided by criteria weight for computation of weighted priority. In case of flexibility criteria, weighted priority of alternative 1 equal to (2.82 / 0.420) = 6.722. In the same manner, weighted priority is calculated for all criteria and alternatives. Subsequently, averaging the results of each row is done which gives the maximum eigenvalue (λ max). For flexibility, λ max is calculated as (6.722 + 6.867 + 6.618 + 6.269 + 6.240 + 6.134) / 6 = 6.475. Similarly, λ max for the remaining three qualitative measures are computed and listed in Table 16.

Table 16
Criteria and respective eigenvalues (λ max)

Criteria	Flexibility	Accessibility	Maintenance	Human Issue
λ max	6.475	6.383	6.354	6.465

The Consistency Index (CI) is expressed by Eq. (2).

$$(\mathrm{CI}) = \frac{(\lambda_{\mathrm{Max}} - \mathrm{n})}{(\mathrm{n} - 1)} \tag{2}$$

where n is the number of compared alternatives. In case of flexibility, λmax is 6.475 and n is 6. Using Equation 2, CI equals to (6.475 - 6) / (6 - 1) = 0.095. Similarly, for remaining criteria, CI are computed (Table 17).

Table 17
Criteria and respective CI values

Criteria	Flexibility	Accessibility	Maintenance	Human Issue
CI	0.095	0.077	0.071	0.093

Consistency Ratio (CR) is computed by

$$(\mathrm{CR}) = \frac{(\mathrm{CI})}{(\mathrm{RI})} \tag{3}$$

Random index (RI) for different attributes (n) are listed in Table 18. When CR is greater than 0.1, this procedure is repeated to improve consistency (Hadi-vencheh & Mohamadghasemi, 2013).

Table 18
Random Index values for different values of n

n	3	4	5	6	7	8	9	10
RI	0.58	0.9	1.12	1.24	1.32	1.41	1.45	1.49

For example, CR for flexibility is equal to 0.095/1.24 = 0.077 and thus the CR values for all the criteria are computed and are listed in Table 19.

Table 19
Criteria and CR values

Criteria	Flexibility	Accessibility	Maintenance	Human Issue
CR	0.077	0.062	0.057	0.075

Since all CR values are not greater than 0.1, the findings are acceptable and show the goodness of the decisions. The values of quantitative performance measures are measured from layout designs discussed in previous section 3. The values of quantitative and qualitative measures under consideration are reported in Table 20.

Table 20
Performance measures values for layout alternatives

Layout Alternative	Quantitative Performance		Qualitative Performance			
	Flow Distance	Adjacency Score	Flexibility	Accessibility	Maintenance	Human Issue
A1	816.76	616	0.224	0.106	0.026	0.056
A2	834.17	599	0.133	0.074	0.018	0.039
A3	875.81	598	0.088	0.040	0.010	0.021
A4	777.69	616	0.019	0.015	0.002	0.006
A5	739.07	553	0.038	0.009	0.005	0.005
A6	900.88	597	0.032	0.019	0.004	0.010

5. Optimum layout design

For the selection of optimum layout design, MCDM methods namely TOPSIS and WASPAS are applied this case for determining the best solution amongst these six alternatives.

5.1 TOPSIS method coupled with Entropy method

For finding out the positive ideal solution and the negative ideal solution in the TOPSIS method the procedure mentioned by Behzadian et al. (2012) is adopted. After forming an initial decision matrix (Table 20), each element is normalized by Eq. (4).

$$r_{ij} = \frac{x_{ij}}{\sqrt{\Sigma x_{ij}^2}} \qquad \text{For } i = 1, \ldots\ldots, m;\ j = 1, \ldots\ldots, n \tag{4}$$

where x_{ij} and r_{ij} are the original and normalized values. For example, the normalised value of flow distance for alternative No.1 is calculated as,

$$r_{ij} = \frac{816.76}{\sqrt{816.76^2 + 834.17^2 + 875.81^2 + 777.69^2 + 739.07^2 + 900.88^2}} = 0.4037330$$

For remaining alternatives and criteria, normalized values are computed and reported in Table 21.

Table 21
Normalized decision matrix for TOPSIS

Alternative	Flow Distance	Adjacency Score	Flexibility	Accessibility	Maintenance	Human Issue
A1	0.4037330	0.4213320	0.8000000	0.7681159	0.7647059	0.7671233
A2	0.4123390	0.4097043	0.4750000	0.5362319	0.5294118	0.5342466
A3	0.4329221	0.4090203	0.3142857	0.2898551	0.2941176	0.2876712
A4	0.3844203	0.4213320	0.0678571	0.1086957	0.0588235	0.0821918
A5	0.3653301	0.3782412	0.1357143	0.0652174	0.1470588	0.0684932
A6	0.4453144	0.4083364	0.1142857	0.1376812	0.1176471	0.1369863

The weighted normalized decision matrix (v_{ij}) is given by Eq. (5).

$$v_{ij} = w_j r_{ij} \tag{5}$$

Weights of individual criteria are found by Entropy method (applied by Chen et al., 2014 for food industry). Normalizing decision matrix (Table 21) is again normalized by using Eq. (6).

$$r_{ij} = \frac{x_{ij}}{\sum_{i=1}^{m}(x_{ij})} \tag{6}$$

where x_{ij} and r_{ij} are the initial and normalized values. For example for cell (1,1) the value by entropy method is equal to (0.4037330) / (0.4037330 + 0.4123390 + 0.4329221 + 0.3844203 + 0.3653301 + 0.4453144) = 0.1651896. In this way the other values mentioned in the Table 22 are calculated.

Table 22
Normalized decision matrix for Entropy method

Alternative	Flow Distance	Adjacency Score	Flexibility	Accessibility	Maintenance	Human Issue
A1	0.1651896	0.1721151	0.4194757	0.4030418	0.4000000	0.4087591
A2	0.1687107	0.1673652	0.2490637	0.2813688	0.2769231	0.2846715
A3	0.1771324	0.1670858	0.1647940	0.1520913	0.1538462	0.1532847
A4	0.1572877	0.1721151	0.0355805	0.0570342	0.0307692	0.0437956
A5	0.1494768	0.1545124	0.0711610	0.0342205	0.0769231	0.0364964
A6	0.1822028	0.1668064	0.0599251	0.0722433	0.0615385	0.0729927

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After normalization, next step is to calculate entropy value (e_j) which is calculated using Eq. (7).

$$e_j = -h \sum_{i=1}^{m} r_{ij} \text{In} r_{ij} \tag{7}$$

where, h = 1/ln (m) and m is number of criteria. Here m is 6, therefore h = 1/ln (6) = 0.5581. Using Eq. (7), entropy value of flow distance equal to -0.5581 × [(0.1651896 + ln 0.1651896) + (0.1687107 + ln 0.1687107) + (0.1771324 + ln 0.1771324 + (0.1572877 + ln 0.1572877) + (0.1494768 + ln 0.1494768) + (0.1822028+ ln 0.1822028)] = 0.9987357 and similarly other entropy values are computed and are summarized in Table 23.

Table 23
Entropy values

Criteria	Flow Distance	Adjacency Score	Flexibility	Accessibility	Maintenance	Human Issue
e_j	0.9987357	0.9996279	0.8277693	0.8249606	0.8293663	0.8146639

Weight factor (w_j) for each criteria is determined by Eq. (8).

$$w_j = \frac{1 - e_j}{\sum_{j=1}^{n}(1 - e_j)} \tag{8}$$

According to Table 23, entropy value (e_j) of flow distance is 0.9987357. Using Eq. (8), weight of flow distance is equal to (1 - 0.9987357) / (1 - 0.9987357) + (1 - 0.9996279) + (1- 0.8277693) + (1- 0.8249606) + (1- 0.8293663) + (1- 0.8146639) = 0.0017936 and similarly weight factors as reported in Table 24 are calculated.

Table 24
Weight factors

Criteria	Flow Distance	Adjacency Score	Flexibility	Accessibility	Maintenance	Human Issue
w_j	0.0017936	0.0005279	0.2443418	0.2483265	0.2420762	0.2629343

By considering these weighted factors, the weighted normalized decision matrix along with positive and negative ideal solutions A* and A' (calculated by using Equations 9 and 10) are summarized in Table 25.

$$A^* = \{v_1^*, \dots, v_n^*\}, \text{Where}, v_j^* = \{\max(v_{ij}) \text{ if } j \, \varepsilon \, J; \min(v_{ij}) \text{ if } j \, \varepsilon \, J'\} \tag{9}$$

$$A' = \{v_1', \dots, v_n'\} \quad \text{Where}, v' = \{\min(v_{ij}) \text{ if } j \, \varepsilon \, J; \max(v_{ij}) \text{ if } j \, \varepsilon \, J'\} \tag{10}$$

Table 25
Weighted normalized decision matrix with positive and negative ideal solution

Alternative	Flow Distance	Adjacency Score	Flexibility	Accessibility	Maintenance	Human Issue
A1	0.0007241	0.0002224	0.1954734	0.1907435	0.1851171	0.2017030
A2	0.0007422	0.0002163	0.1160624	0.1331606	0.1281580	0.1404717
A3	0.0007793	0.0002159	0.0767931	0.0719787	0.0711989	0.0756386
A4	0.0006920	0.0002224	0.0165803	0.0269920	0.0142398	0.0216110
A5	0.0006576	0.0001997	0.0331607	0.0161952	0.0355994	0.0180092
A6	0.0008016	0.0002156	0.0279248	0.0341899	0.0284796	0.0360184
A*	0.0006576	0.0002224	0.1954734	0.1907435	0.1851171	0.2017030
A'	0.0008016	0.0001997	0.0165803	0.0161952	0.0142398	0.0180092

The separation from the positive ideal alternative is given by

$$S_i^* = [\Sigma(v_j^* - v_{ij})^2]^{\frac{1}{2}} \; i = 1, \dots, m \tag{11}$$

The separation from the negative ideal alternative is given by

$$S_i' = [\Sigma(v_j' - v_{ij})^2]^{\frac{1}{2}} \; i = 1, \dots., m \tag{12}$$

For alternative No.1, separation of positive ideal solution is equal to [(0.0007241 - 0.0006576) ^2 + (0.0002224 - 0.0002224) ^2 + (0.1954737- 1954734) ^2 + (0.1907435 - 0.1907435) ^2 + (0.1851171 - 0.1851171) ^2 + (0.2017030 - 0.2017030) ^2] ^0.5 = 0.0000844 and the entire results are shown in Table 26. Also, the relative closeness coefficient of each alternative is calculated by using Eq. (13).

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$$C_i^* = S_i' / (S_i^* + S_i'), \quad 0 < C_i^* < 1 \tag{13}$$

For alternative No.1, (C_i^*) is equal to 0.3541389 / (0.0000844 + 0.3541389) = 0.9997618 and these results are also shown in Table 26.

Table 26
Separation measures of positive and negative ideal solutions, relative closeness and rank

Alternative	Separation measure of positive ideal solution (S_i^*)	Separation measure of negative ideal solution (S_i')	Relative closeness (C_i^*)	Rank
A1	0.0000844	0.3541389	0.9997618	1
A2	0.1288671	0.2270534	0.6379329	2
A3	0.2388398	0.1153409	0.3256555	3
A4	0.3470268	0.0113847	0.0317644	6
A5	0.3359901	0.0270407	0.0744859	5
A6	0.3233384	0.0313014	0.0882626	4

Larger relative closeness (C_i^*) value shows the optimum alternative for all the performance measures under consideration. So from Table 26, it is clear that alternative 1 is having maximum relative closeness value and hence is the optimum solution of TOPSIS coupled with entropy method.

5.2 WASPAS method coupled with Entropy method

WASPAS method is a unique combination Weighted Sum Method (WSM) and Weighted Product Method (WPM) and is useful to improve ranking accuracy. The application of WASPAS method (illustrated by Chakraborty & Zavadskas, 2014 for manufacturing decision making) is applied in this case as follows. For the initial decision matrix (Table 20), normalization of beneficial and non-beneficial criteria are done. For beneficial criteria,

$$r_{ij} = \frac{x_{ij}}{\max_i x_{ij}} \quad i = 1,2,\ldots,m \text{ and } j = 1,2,\ldots,n \tag{14}$$

For non-beneficial criteria,

$$r_{ij} = \frac{\min_i x_{ij}}{x_{ij}} \quad = 1,2,\ldots,m \text{ and } j = 1,2,\ldots,n \tag{15}$$

where x_{ij} and r_{ij} are the original and normalized values. Flow distance is non beneficial criteria and its minimum value is 739.07 which is for alternative No. 5. For example, the normalized value of alternative No. 1 is equal to 739.07/816.76 = 0.9048803. Similarly, normalized values for remaining performance measures and alternative are computed and listed in Table 27.

Table 27
Normalised decision matrix for WASPAS method

Alternative	Flow Distance	Adjacency Score	Flexibility	Accessibility	Maintenance	Human Issue
A1	0.9048803	1.0000000	1.0000000	1.0000000	1.0000000	1.0000000
A2	0.8859945	0.9724026	0.5937500	0.6981132	0.6923077	0.6964286
A3	0.8438702	0.9707792	0.3928571	0.3773585	0.3846154	0.3750000
A4	0.9503401	1.0000000	0.0848214	0.1415094	0.0769231	0.1071429
A5	1.0000 000	0.8977273	0.1696429	0.0849057	0.1923077	0.0892857
A6	0.8203867	0.9691558	0.1428571	0.1792453	0.1538462	0.1785714

The total relative importance of the i_{th} alternative based on WSM is calculated using Eq. (16).

$$Q_i^{(1)} = \sum_{j=1}^{n} r_{ij} w_j \tag{16}$$

where, w_j is weight of j_{th} criteria. As reported earlier, criteria weight (w_j) obtained by Entropy method is employed. By considering the weights calculated for entropy method (Table 24), normalised value of flow distance for alternative No.1 is 0.9048803 × 0.0017936 = 0.0016230 and performance score ($Q_i^{(1)}$) is equal to (0.0016230 + 0.0005279 + 0.2443418 +

0.2483265 + 0.2420762 + 0.2629343) = 0.9998297. Similarly for remaining alternatives weighted normalised decision matrix and performance scores are computed (Table 28).

Table 28
Weighted normalised decision matrix and performance score of WSM

Alternative	Flow Distance	Adjacency Score	Flexibility	Accessibility	Maintenance	Human Issue	$Q_i^{(1)}$
A1	0.0016230	0.0005279	0.2443418	0.2483265	0.2420762	0.2629343	0.9998297
A2	0.0015891	0.0005133	0.1450779	0.1733600	0.1675912	0.1831150	0.6712466
A3	0.0015136	0.0005125	0.0959914	0.0937081	0.0931062	0.0986004	0.3834322
A4	0.0017045	0.0005279	0.0207254	0.0351405	0.0186212	0.0281715	0.1048912
A5	0.0017936	0.0004739	0.0414508	0.0210843	0.0465531	0.0234763	0.1348321
A6	0.0014714	0.0005116	0.0349060	0.0445114	0.0372425	0.0469526	0.1655954

The total relative importance of the i_{th} alternative based on WPM is calculated by using Eq. (17).

$$Q_i^{(2)} = \prod_{j=1}^{n} (r_{ij})^{w_j} \tag{17}$$

The weighted normalised value of flow distance for alternative No.1 is 0.9048803 ^ 0.0017936 = 0.9998207 and performance score ($Q_i^{(2)}$) is equal to (0.9998207 × 1.000000 × 1.000000 × 1.000000 × 1.000000 × 1.000000) = 0.9998207. Accordingly weighted normalised decision matrix values and performance scores are shown in Table 29.

Table 29
Weighted normalized decision matrix and Performance score of WPM

Alternative	Flow Distance	Adjacency Score	Flexibility	Accessibility	Maintenance	Human Issue	$Q_i^{(2)}$
A1	0.9998207	1.0000000	1.0000000	1.0000000	1.0000000	1.0000000	0.9998207
A2	0.9997829	0.9999852	0.8804038	0.9146241	0.9148296	0.9092574	0.6696547
A3	0.9996956	0.9999843	0.7958928	0.7850491	0.7934962	0.7726774	0.3829618
A4	0.9999086	1.0000000	0.5472542	0.6153433	0.5374534	0.5558327	0.1005893
A5	1.0000000	0.9999430	0.6482512	0.5420341	0.6709229	0.5298154	0.1248942
A6	0.9996450	0.9999835	0.6215946	0.6525462	0.6356426	0.6357344	0.1638498

A joint generalized equation for determining the total relative importance of criteria (Qi) is displayed in Eq. (18).

$$Q_i = 0.5Q_i^{(1)} + 0.5\,Q_i^{(2)} \tag{18}$$

For Alternative No.1, Q_i = (0.5 × 0.9998297) + (0.5 × 0.9998207) = 0.9998252. Table 30 summarizes values of total relative importance for all the alternatives.

Table 30
Total relative importance

Alternative	$Q_i^{(1)}$	$Q_i^{(2)}$	Q_i	Rank
A1	0.9998297	0.9998207	0.9998252	1
A2	0.6712466	0.6696547	0.6704507	2
A3	0.3834322	0.3829618	0.3831970	3
A4	0.1048912	0.1005893	0.1027402	6
A5	0.1348321	0.1248942	0.1298632	5
A6	0.1655954	0.1638498	0.1647226	4

According to Table 30, alternative 1 has the highest rank, so it gives the best multiple performance characteristics and is taken as optimum solution of WASPAS method. To increase ranking accuracy and effectiveness more generalized Eq. (19) is used. Total relative importance of i_{th} alternative is found by,

$$Q_i = \lambda Q_i^{(1)} + (1-\lambda)Q_i^{(2)} \tag{19}$$

where, λ = 0, 0.1……1. For example λ = 0.9, total relative importance (Qi) of alternative No.1 is equal to (0.9 × 0.9998297) + ((1 - 0.9) × 0.9998207) = 0.9998288. Similarly for remaining alternatives total relative importance for different values of λ is as shown in Table 31.

Table 31
Ranking of alternatives for different λ values

Alternative	λ = 0	λ = 0.1	λ = 0.2	λ = 0.3	λ = 0.4	λ = 0.5	λ = 0.6	λ = 0.7	λ = 0.8	λ = 0.9	λ = 1
A1	0.9998207	0.9998216	0.9998225	0.9998234	0.9998243	0.9998252	0.9998261	0.9998270	0.9998279	0.9998288	0.9998297
A2	0.6696547	0.6698139	0.6699731	0.6701323	0.6702915	0.6704507	0.6706098	0.6707690	0.6709282	0.6710874	0.6712466
A3	0.3829618	0.3830088	0.3830558	0.3831029	0.3831499	0.3831970	0.3832440	0.3832910	0.3833381	0.3833851	0.3834322
A4	0.1005893	0.1010195	0.1014497	0.1018799	0.1023101	0.1027402	0.1031704	0.1036006	0.1040308	0.1044610	0.1048912
A5	0.1248942	0.1258880	0.1268818	0.1278756	0.1288694	0.1298632	0.1308569	0.1318507	0.1328445	0.1338383	0.1348321
A6	0.1638498	0.1640243	0.1641989	0.1643735	0.1645480	0.1647226	0.1648972	0.1650717	0.1652463	0.1654209	0.1655954

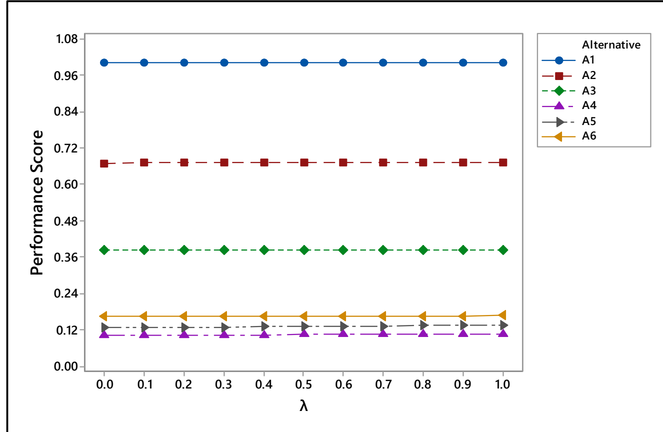

Fig. 12. Variation of performance scores for different λ values

Fig. 12 highlights the effect of the varying values of λ on the performance scores and ranking in WASPAS method. It is interesting to note that the ranking of all alternatives remains constant over the considered range of λ values.

6. Rank Reversal study

The objective of this section is to study the rank reversal in TOPSIS by numerical analysis. The effect of addition of an alternative on the ranking order is checked. There are six alternatives, each of which has six attributes. The initial and combination values for the alternatives and the ranking results are reported in Table 32. It is clear that, when alternative 4 i.e. layout having a lower rank is eliminated, then the ranking of the five old alternatives becomes A5 > A1 > A2 > A3 > A6. When the new alternative A7 is added to the previous six alternatives, the first rank does not change but remaining ranks get changed and it becomes A1 > A2 > A7 > A3 > A6 > A5 >A4. In the above two combinations, different rankings are obtained as compared to the original one. So vector normalization affects the independence between alternatives which disturbs the initial ranking. To overcome this, it is necessary to apply normalization method which maintain independence between alternatives by keeping the ideal solution constant. Considering the above condition, the Max-Min normalization method is used and the results are reported in Table 33.

Table 32
Initial and combination values by considering Vector normalization method

	Layout	Flow Distance /Cost-1	Adjacency Score	Flexibility	Accessibility	Maintenance	Human Issue	Rank
Initial layout	A1-SLP	816.76	616	0.224	0.106	0.026	0.056	1*
	A2-S	834.17	599	0.133	0.074	0.018	0.039	2
	A3-U	875.81	598	0.088	0.04	0.01	0.021	3
	A4-ALDEP	777.69	616	0.019	0.015	0.002	0.006	6
	A5-CRAFT	739.07	553	0.038	0.009	0.005	0.005	5
	A6-CORELAP	900.88	597	0.032	0.019	0.004	0.010	4
Delete Layout 4	A1-SLP	816.76	616	0.224	0.106	0.026	0.056	2
	A2-S	834.17	599	0.133	0.074	0.018	0.039	3
	A3-U	875.81	598	0.088	0.04	0.01	0.021	4
	A5-CRAFT	739.07	553	0.038	0.009	0.005	0.005	1*
	A6-CORELAP	900.88	597	0.032	0.019	0.004	0.010	5
Addition of Layout 7	A1-SLP	816.76	616	0.224	0.106	0.026	0.056	1*
	A2-S	834.17	599	0.133	0.074	0.018	0.039	2
	A3-U	875.81	598	0.088	0.04	0.01	0.021	4
	A4-ALDEP	777.69	616	0.019	0.015	0.002	0.006	7
	A5-CRAFT	739.07	553	0.038	0.009	0.005	0.005	6
	A6-CORELAP	900.88	597	0.032	0.019	0.004	0.010	5
	A7	824.06	596.50	0.089	0.044	0.011	0.023	3

Table 33
Initial and other combination values by considering Max-Min normalization method

	Layout	Flow Distance /Cost-1	Adjacency Score	Flexibility	Accessibility	Maintenance	Human Issue	Rank
Initial layout	A1-SLP	816.76	616	0.224	0.106	0.026	0.056	1*
	A2-S	834.17	599	0.133	0.074	0.018	0.039	2
	A3-U	875.81	598	0.088	0.04	0.01	0.021	3
	A4-ALDEP	777.69	616	0.019	0.015	0.002	0.006	6
	A5-CRAFT	739.07	553	0.038	0.009	0.005	0.005	5
	A6-CORELAP	900.88	597	0.032	0.019	0.004	0.010	4
Delete Layout 4	A1-SLP	816.76	616	0.224	0.106	0.026	0.056	1*
	A2-S	834.17	599	0.133	0.074	0.018	0.039	2
	A3-U	875.81	598	0.088	0.04	0.01	0.021	3
	A5-CRAFT	739.07	553	0.038	0.009	0.005	0.005	5
	A6-CORELAP	900.88	597	0.032	0.019	0.004	0.010	4
Addition of Layout 7	A1-SLP	816.76	616	0.224	0.106	0.026	0.056	1*
	A2-S	834.17	599	0.133	0.074	0.018	0.039	2
	A3-U	875.81	598	0.088	0.04	0.01	0.021	4
	A4-ALDEP	777.69	616	0.019	0.015	0.002	0.006	7
	A5-CRAFT	739.07	553	0.038	0.009	0.005	0.005	6
	A6-CORELAP	900.88	597	0.032	0.019	0.004	0.010	5
	A7	824.06	596.50	0.089	0.044	0.011	0.023	3

From Table 33, it is observed that the first rank remains unchanged for all cases which ensures good agreement in the decision results.

7. Conclusion

This paper addresses the real-life problem of the gearbox manufacturing industry. Six alternative layout designs are developed by using SLP, S flow pattern, U flow pattern, ALDEP, CRAFT, and CORELAP methods. For choosing the optimum configuration, we considered quantitative and qualitative performance measures. Quantitative performance is directly measured from layout design whereas AHP method is applied for getting the qualitative performance data. For layout designs, all the criteria do not have equal impact, therefore it is not possible to assign equal weights to these performance measures. For this purpose, for calculating the weights, the Entropy method is used. Subsequently, two MCDM approaches TOPSIS and WASPAS both coupled with the entropy method are considered for selection and comparison of the optimum layout design. The ranks of all the alternatives obtained by the TOPSIS method are the same as those obtained by the WASPAS method. Both MCDM approaches give SLP layout as optimum solution. In the case of the WASPAS method, the ranking of all alternatives remains unaffected for λ values which confirms the optimum solution developed by SLP. Also, rank reversal phenomenon is applied to TOPSIS and the causes of occurrence are reported. It has been observed that the Max-Min normalization method is stable and robust compared to the vector normalization method.

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YASHODA TECHNICAL CAMPUS 6757 SATARA
DIRECTOR
Yashoda Technical Campus
Satara

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AN EXPERIMENTAL INVESTIGATION OF LASER TRANSFORMATION HARDENING OF UNALLOYED TITANIUM USING ND:YAG LASER

Dr. Duradundi. Sawant. Badkar
Department of Mechanical Engineering,
Dr. J.J.Magdum College of Engineering, Jaysingpur : 416101, Shivaji University,
Kolhapur, Maharashtra, India.

Abstract—**This research paper presents the investigation on laser transformation hardening (LTH) of unalloyed titanium of 1.6mm thickness sheet, nearer to ASTM Grade 3 of chemical composition was investigated using 2KW CW Nd: YAG Laser. The effects of laser process parameters: laser power750-1250W), scanning speed (1000-3000 mm/min) and focal point position (-10mm to -30mm) on the hardened bead profile parameters such as hardened bead width (HBW) and hardened depth (HD) was investigated using Response Surface Methodology (RSM). The experimental design is based on an independent quadratic Box-Behnken Design (BBD) matrix. The reduced linear and quadratic polynomial equations for predicting hardened bead width and hardened depth of bead profile were developed. Adequacies of models were examined by Analysis of Variance (ANOVA) technique. The results designate that the mathematical models developed predict the responses adequately adequate within the limits of hardening parameters being employed. It is recommended that the regression equations can be used to find optimum laser hardening conditions for desired criterion.**

Keywords: **Laser transformation hardening; response surface methodology; full factorial design; analysis of variance; bead geometry.**

I. INTRODUCTION

Titanium is a very attractive material for aerospace applications due to its low thermal conductivity, relatively low density and elastic modulus, light weight, high strength to weight ratio and temperature capabilities; titanium is used in cryogenic applications as well as for elevated temperature applications up to ~600°C. Titanium and its alloys are of excellent corrosion resistance, wear resistance, and high specific strength (strength/density), titanium and its alloys have been extensively used for chemical, electric power, marine, biomedical, pharmaceutical and aerospace industries as major metal materials by taking advantage of their characteristics. On the other hand, their applications to automobile industry have been limited except for racing cars and special-purpose cars because of their high cost despite the strong interest shown in titanium materials by the industry in terms of lightweight, fuel efficiency and performances. A unique aspect of titanium is that even the modulus can be modified by heat treatment and/or processing. The laser surface transformation hardening was initially reported in the early seventies and that has become an established technology in manufacturing and processing industries to improve the surface characteristics of intricate and selected areas of various engineering components. This has been revolutionizing automobile and aerospace industries for hardening surface layers of the turbine blades, crankshafts, piston ring grooves and tractor engine components, etc. Laser hardening process gives a wear-resistant surface layer, thereby increasing the service life of components to a considerable extent. All cast iron, medium-carbon steel and tool steel are amenable to the laser hardening process [1].

Laser Surface Transformation Hardening (LSTH) fundamentally allows obtaining a hardened surface layer in titanium and its alloys by changing the base structure into hardened transformed beta marten site. Harden ability of titanium and its alloys is a phrase that refers to its ability to permit full transformation of the titanium and its alloys to transformed beta (marten sites, alpha) or to retain beta to room temperature. The standard laser transformation hardening (LTH) of titanium and its alloys involves two main steps: 1. beta phase formation, in which the material is heated to/above the beta transus temperature, i.e., β-transus (88 ˚C or 1621˚F), in order to form the material with 100% beta phase (but below the melting point) and 2. "self quenching" or cooling down, where β-phase is transformed into harder acicular (plate-like) α marten site (transformed β) or retain beta to room temperature. The β-transus is defined as the lowest equilibrium temperature at which the

material is 100% beta or alpha, which does not exist. The β-transus is critical in deformation processing and in heat treatment. A correct treatment requires the heating stage long enough for the β-phase formation to complete and allow the alloying elements such as manganese, carbon, oxygen and nitrogen to stabilize it and dissolve iron, vanadium, molybdenum, copper, nickel and silicon into the matrix. Self quenching should be fast enough so as to suppress the normal breakdown of β-phase into the initial α or (α+β) phases and produce hard plate like alpha marten site formation instead [2].

A high power laser beam can be delivered to the work piece with a very precise spatially and temporally control. For the localized laser heating, bulk of the work piece remains at a low temperature and acts as a heat sink and therefore, when laser irradiation is stopped, rapid self-quenching takes place. Compared to standard hardening procedures, the laser techniques offer several advantages. The applied laser radiation instantaneously heats a localized region on the surface and the bulk of the material acts as an efficient heat sink producing high cooling rates [3, 4]. This means that desired hardened depths can be achieved at high laser processing speeds and with minimal thermal distortion in the treated parts. In addition, it has been shown that laser surface hardening not only increase the wear and corrosion resistance but also increase the fatigue strength under certain conditions. [5, 6]. In this research, continuous wave 2kW, Nd: YAG laser source with radiation wavelength of 1.064 µm has been used to produce hardening of commercially pure titanium nearly ASTM Grade 3 of 1.6mm thickness sheet.

From the literature survey it has been observed that many authors have published their research work related to only laser welding, cladding, cutting, hardening processes specifically steel materials with full factorial design, Box–Behnken design, Plackett-Burman design, and Central Composite Design using RSM. Prachya Peasura studied the application of the Response Surface Methodology (RSM) and Central Composite Design (CCD) experiment in mathematical model and optimizes post weld heat treatment (PWHT). Author found the influence of PWHT and the most appropriate mathematical model as the basis for further ASTM A516 grade 70 weld applications using response surface methodology (RSM) to find the optimal parameters and the central composite design (CCD) experimental design for a mathematical model to predict the tensile strength [7].D. S. Badkar, et al. investigated the influence of laser phase transformation hardening parameters on heat input and hardened-bead profile quality of unalloyed titanium using Nd: YAG laser. The applied the response surface methodology (RSM) in order to develop the mathematical modeling of heat input and angle of entry of hardened bead geometry [1]. Duradundi Sawant Badkar et al. investigated and evaluated the optimal values of laser process parameters: laser power, scanning speed, and focused position for the simultaneous minimization and maximization of heat input and tensile strength respectively by Taguchi method and utility concept approach in laser transformation hardening of commercially pure titanium sheet of 1.6mm thickness using continuous wave (CW) Nd: YAG laser beam [8]. Duradundi Sawant Badkar employed a Full Factorial Design (FFD) with Response Surface Methodology(RSM)is to establish, optimize and to investigate the relationships of three laser transformation hardening process parameters: laser power, scanning speed, and focused position on laser hardened bead profile parameters such as angle of entry of hardened bead profile and power density. RSM is used to develop pseudo-closed-form models from the computational parametric studies. The results demonstrate that the developed models are accurate with low percentages of error [9]. Duradundi Sawant Badkar studied the effect of laser process variables such as laser power, scanning speed, and focused position was investigated using response surface methodology (RSM) and artificial neural network (ANN) keeping argon gas flow rate of 10 lpm as fixed input parameter. This paper describes the comparison of the heat input (HI) and ultimate tensile strength (σ) (simply called as tensile strength) predictive models based on ANN and RSM. The paper also presents the effect of laser process variables on the HI and ultimate σ. The research work also emphasizes on the effect of HI on σ. The experiments were conducted based on a three-factor, three-level Box-Behnken surface statistical design. Quadratic polynomial equations were developed for proper process parametric study for its optimal performance characteristics [10].

Duradundi Sawant Badkar et al. in their study applied the Response Surface Methodology (RSM) and Central Composite Design (CCD) for modeling, optimization, and an analysis of the influences of dominant laser-processing parameters namely: laser power (LP), scanning speed (SS), and focused position (FP) on heat input (HI) and hardened bead geometries such as hardened bead width (HBW), hardened depth (HD), angle of entry of hardened bead profile (AEHB) of laser transformation hardened surface quality of commercially pure titanium sheet of 1.6 mm in thickness using continuous wave (CW) 2-kW Nd:YAG laser. They showed the predicted results are compared with the experimental results and are good agreement with heat input and hardened bead profile parameters [11]. Duradundi Sawant Badkar et al. developed the mathematical models from Response Surface Methodology (RSM) are used to predict the laser phase transformation hardened bead profile parameters in terms of the laser process factors; namely laser power (LP), scanning speed (SS) and focused position (FP), used to optimize the laser transformation hardening process. It has been investigated that the results accomplished from both numerical optimization and graphical methods ensured almost analogous values to each other for optimal LTH conditions in all the cases [12].

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In this research article author has made an effort in establishing the influence of laser process parameters on hardened bead geometry, and developing the mathematical models in order to optimize the laser process parameters thereby achieving the hardened bead geometry with maximum hardened bead width and minimum hardened depth.

II. EXPERIMENTAL DESIGN

The experimental design is based on a three level Box-Behnkin design with full replication [13]. Box-Behnkin designs are response surface designs specially made to require only 3 levels, codes as -1, 0, and +1. Table 1 shows laser input variables and experimental design levels used.

A response surface method (RSM) has been often applied to optimize the formulation variables [14, 15]. RSM designs allow us to estimate interaction and even quadratic effects, and hence give us an idea of the (local) shape of the response surface under investigation. The optimization procedure based on RSM includes statistical experimental designs, multiple regression analysis, and mathematical optimization algorithms for seeking the best formulation under a set of constrained equations. RSM was applied to the experimental data using statistical software, Design-expert 7. Linear and second order polynomials were fitted to the experimental data to obtain the regression equations. The sequential F-test, lack-of –fit test and other adequacy measures were used in selecting the best models. A step-wise regression method was used to fit the second order polynomial equation (1) to the experimental data and to indentify the relevant model terms [16, 17]. The same statistical software was used to generate the statistical and response plots. The second-order model is utilized to find a suitable approximation for the functional relationship between independent variables and the response surface.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_{ii} + \sum_{i=1} \sum_{j=i+1} \beta_{ij} X_i X_j + \varepsilon \quad (1)$$

where Y is the response calculated by model (dependent variables), β_0 is the constant coefficient, β_i are the coefficients for the linear effect, β_{ii} are the coefficients for the quadratic effect, β_{ij} are the coefficients for the cross-product effect, Xij, Xj are the variables corresponding to factors (independent variables), ε is the error, k the number of variables considered and i, j are the factors.

III. EXPERIMENTAL METHODOLOGY

The experiments are conducted on a given unalloyed Titanium alloy substrate with chemical composition given in Table 2. The chemistry is nearer to ASTM Gr. 3. The thickness of the substrate selected is 1.6 mm, to simulate the majority of the industrial applications that is in practice at present. For conducting the experiments on the substrate, the materials surface is cleaned properly with suitable agents.

TABLE 1. PROCESS TITANIUM VARIABLES AND EXPERIMENTAL DESIGN LEVELS USED

Variables	-1	0	+1
Laser power, LP(Watts)	750	1000	1250
Scanning speed, SS (mm/min)	1000	2000	3000
Focused position, FP (mm)	-30	-20	-10

TABLE 2. CHEMICAL COMPOSITION OF UNALLOYED

Ele.	Ti	C	Fe	Mo	V	Cu	O	Al
% By Wt	Bal.	0.011	0.15	0.003	0.029	0.14	0.1	1.1

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TABLE 3. DESIGN MATRIX WITH CODE INDEPENDENT PROCESS VARIABLES

Exp No	Run order	Coded variables			Actual variables		
		LP (Watts)	SS (mm/ min)	FP (mm)	LP (Watts)	SS (mm/ min)	FP (mm)
1	14	-1	-1	0	750	1000	-20
2	1	1	-1	0	1250	1000	-20
3	4	-1	1	0	750	3000	-20
4	8	1	1	0	1250	3000	-20
5	3	-1	0	-1	750	2000	-30
6	5	1	0	-1	1250	2000	-30
7	6	-1	0	1	750	2000	-10
8	16	1	0	1	1250	2000	-10
9	10	0	-1	-1	1000	1000	-30
10	13	0	1	-1	1000	3000	-30
11	7	0	-1	1	1000	1000	-10
12	15	0	1	1	1000	3000	-10
13	12	0	0	0	1000	2000	-20
14	11	0	0	0	1000	2000	-20
15	9	0	0	0	1000	2000	-20
16	17	0	0	0	1000	2000	-20
17	2	0	0	0	1000	2000	-20

Figure 1. Solid state Nd: YAG Laser source at WRI used for experimental work [18].

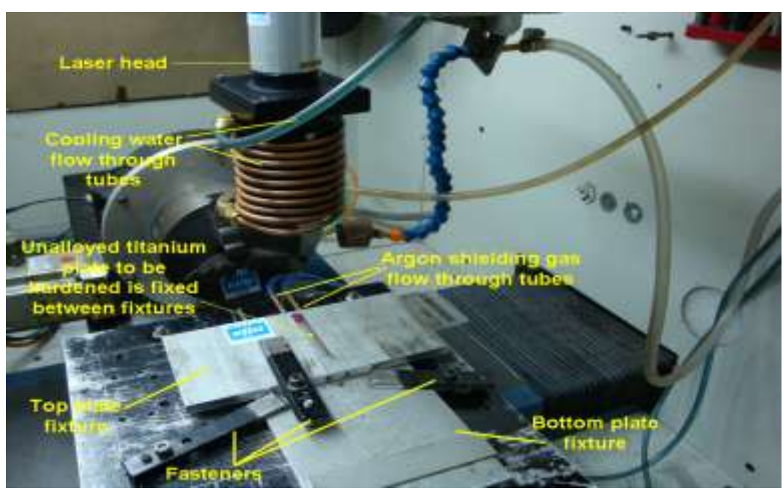

Figure 2. Experimental set-up showing the laser beam head and shielding arrangements in the working chamber [18].

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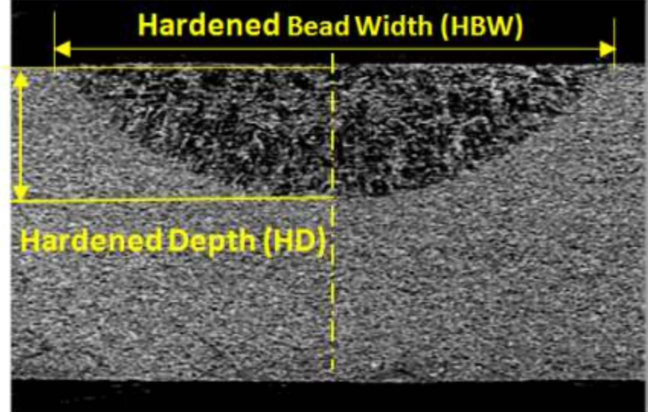

Figure. 3. Microstructure of Hardened - Bead Profile with parameters, HBW, HD and AEHB

A continuous wave (CW) 2KW, with radiation wavelength λ=1.06µm Nd: YAG laser source from GSI Lumonics is employed for the experimental work as shown in Fig.1. The experiment was carried out according to the design matrix in a random order to avoid any systematic error. A spherical beam configuration is used throughout for the study. The experiment set up is shown in Fig.2. The laser beam is transported through a fibre optic cable to the work centre. Siemens 802 CNC controller is providing the process control during the experiments. The work centre is having x, y and rotational movement for processing applications. The laser source, work centre and the controls are interfaced. Cooling is ensured by a chiller and a cooling tower. For the study, 120mm focal optic is employed with varying beam spot size depending on defocus distance to obtain a wider scan area. Argon gas is employed as shielding medium with a constant flow rate of 8lpm throughout the experimental work. Transverse sectioned specimens were cut from laser hardened bead-on trial of unalloyed titanium sheet and mounted. Standard metallographic was made for each transverse sectioned specimens. The bead profile parameters 'responses' were measured using an optical microscope (with Image processing computer controlled software) with digital micrometers attached to it with an accuracy of 0.001 mm, which allow to measure in x-axes and y-axes directional movement.

Fig.3 shows microstructure of laser Hardened-Bead Profile with measured parameters, such as hardened bead width (HBW), hardened depth (HD) and angle of entry of hardened bead profile(AEHB) for CW spherical beam. The measured laser hardened bead profile parameters 'responses' were recorded. The design matrix is shown in Tables 3.

IV. RESULTS AND DISCUSSION

The results of the laser hardened-bead on trials were measured according to the design matrix with coded and actual independent process variables in Table 3 using the transverse sectioned specimens and the optical microscope mentioned earlier, the measured responses are listed in Table 4. Analyzing the measured responses by the Design-expert software, the fit summary output indicates that the linear model is significantly significant for hardened bead width (HBW) 'the second response' therefore it will be used for further analysis. While for the other response hardened depth (HD) the quadratic models is statistically and is recommended for the further analysis.

A. Analysis of variance (ANOVA)

The adequacy of the developed models were tested using the analysis of variance (ANOVA) technique and the results of the linear and quadratic order response surface model fitting in the form of analysis of variance(ANOVA) are given in Tables 4-5. The test for significance of the regression models, the test for significance on individual model coefficients and the lack-of-fit test were performed using the same statistical Design-expert 7 software package. By selecting the step-wise regression method, which eliminates the insignificant model terms automatically, the resulting ANOVA Tables 4-5 for the response surface quadratic models summarize the analysis of variance of each response and show the significant model terms.

The same Tables show also the other adequacy measures R^2, adjusted R^2 and predicted R^2.The coefficient of determination R^2 indicates the goodness of fit for the model. In this case, all the values of coefficient of determination R^2 are nearly equal to 1. Clearly, we must have $0 \leq R^2 \leq 1$, with larger values being more desirable. The adjusted coefficient of determination R^2 or "adjusted" R^2 is a variation of the ordinary R^2 statistic that reflects the number of factors in the model. The entire adequacy measures are closer to 1, which is in reasonable agreement and indicate adequate models. The adequate precision "Adeq Precision" compares the range of the predicted value at the design points to the average prediction error. Adequate precision measures signal to nose ratio. A ratio greater than 4 is desirable. In all cases the value of adequate precision are dramatically

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greater than 4. The adequate precision ratio above 4 indicates adequate model discrimination. The ANOVA Tables 5-6 also shows the model terms standard Std. Dev, Mean, C.V and PRESS. "Std. Dev." Standard deviation is a square root of the error mean square, ($\sqrt{MS_E}$) and "C.V." is the coefficient of variation, defined ($\sqrt{MS_E}/\bar{y}$) 100, where $\bar{y}$ = Mean. The coefficient of variation, "C.V" measures the unexplained or residual variability in the data as a percentage of the mean of the response variable. At the same time a relatively lower values of the coefficient of variation, C.V., from the Tables 4-5 indicate improved precision and reliability of the conducted experiments. "PRESS" stands for "Prediction Error Sum of Squares," and it is a measure of how well the model for the experiment is likely to predict the responses in a new experiment. Small values of PRESS are desirable. In all the cases the values of PRESS are considerably small. The values of "Probability > F" in Tables 4-5 for all models are less than 0.0500 indicate that all models are significant. In all cases the "Lack-of-fit" values implies the "Lack-of-fit" is not significant relative to the pure error. Non-significant lack- of- fit as it is desired and it is good.

The analysis of variance for the hardened bead width (HBW) model, from the Table 4 the analysis indicated that there is a linear relationship between the main effects of the three process parameters. Also, in the case of hardened depth (HD) model, from the Table 5 the main effect of laser power (LP), scanning speed (SS), focused position (FP), interaction effect of laser power (LP) with scanning speed (SS) and the second order effect of scanning speed (SS) have the significant effect.

TABLE 4. ANOVA TABLE FOR THE HARDENED BEAD WIDTH REDUCED LINEAR MODEL

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1.2231	3	0.4077	157.3093	< 0.0001	Sig.
LP	0.6699	1	0.6699	258.4824	< 0.0001	
SS	0.5045	1	0.5045	194.6655	< 0.0001	
FP	0.0487	1	0.0487	18.78011	0.0008	
Residual	0.0337	13	0.0026			
Lack of Fit	0.0227	9	0.0025	0.922208	0.5804	Not sig.
Pure Error	0.011	4	0.0027			
Corrected Total	1.2568	16				

Std. Dev.	0.0509	R-Squared	0.9732
Mean	2.2522	Adj R-Squared	0.9670
C.V. %	2.2604	Pred R-Squared	0.9533
PRESS	0.0587	Adeq Precision	43.775

TABLE 5. ANOVA TABLE FOR HARDENED DEPTH REDUCED QUADRATIC MODEL

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.9284	5	0.1857	61.4275	< 0.0001	Sig.
LP	0.2309	1	0.2309	76.3746	< 0.0001	
SS	0.6166	1	0.6166	203.989	< 0.0001	
FP	0.0164	1	0.0164	5.4191	0.0400	
LP×SS	0.0195	1	0.0195	6.43796	0.0276	
SS^2	0.0451	1	0.0451	14.9169	0.0026	
Residual	0.0333	11	0.003			
Lack of Fit	0.0153	7	0.0022	0.48566	0.8099	not sig.
Pure Error	0.018	4	0.0045			
Corrected Total	0.9616	16				

Std. Dev.	0.0549	R-Squared	0.9654
Mean	0.6370	Adj R-Squared	0.9497
C.V. %	8.63099	Pred R-Squared	0.9069
PRESS	0.0895	Adeq Precision	27.4010

TABLE 6. CONFIRMATION OF EXPERIMENTS

Exp No	Process parameters	Responses	Actual value	Predicted value	Error	Error %
1	LP=750Watts SS=3000mm/min FP=-30mm	HBW (mm)	1.792	1.78974	0.002	0.112
		HD (mm)	0.259	0.26863	-0.010	-3.861
2	LP=1250Watts SS=2000mm/min FP=-20mm	HBW (mm)	2.425	2.542	-0.117	-4.820
		HD (mm)	0.724	0.758	-0.034	-4.690
3	LP=1250Watts SS=3000mm/min FP=-30mm	HBW (mm)	2.274	2.368	-0.094	-4.130
		HD (mm)	0.440	0.469	-0.029	-6.590
4	LP=1000Watts SS=1000mm/min FP=-20mm	HBW (mm)	2.519	2.503	0.001	0.630
		HD (mm)	1.043	0.969	0.074	7.090
5	LP=750Watts SS=1000mm/min FP=-30mm	HBW (mm)	2.274	2.292	-0.018	-0.791
		HD (mm)	0.716	0.684	0.032	4.470

The final mathematical models in terms of coded factors/variables as determined by design expert software are shown below:

Hardened bead width (HBW) = $2.2522 + 0.2894\times LP - 0.251\times SS - 0.078\times FP$ (2)

Hardened depth (HD) = $0.5884 + 0.1698 \times LP - 0.2776\times SS + 0.0452\times FP - 0.0697\times LP\times SS + 0.1032\times SS^2$ (3)

While the following final empirical models in terms of actual factors/variables:

Hardened bead width (HBW) = $1.441+0.00116\times LP-0.0002511\times SS-0.0078\times FP$ (4)

Hardened depth (HD) = $0.40941+0.00123\times LP-0.00041\times SS+0.00452\times FP-2.79E\text{-}07\times LP\times SS+1.032E\text{-}07\times SS^2$ (5)

The above obtained mathematical models in terms of coded factors/variables (equation. 2 & equation. 3) are related to the coded laser process parameters and empirical models in terms of actual factors/variables (equation. 4 & equation.5) are related to the actual (experimental) values of laser process parameters. By substituting the related values of coded variables and corresponding equivalent values of actual (experimental) variables of laser processing parameters in the mathematical models in terms of coded factors and empirical models in terms of actual factors the corresponding output values of HBW and HD will be almost similar for the developed models of coded and actual factors/variables.

B. Validation of the Models

Figs.4-5 show the relationship between the actual and predicted values of hardened bead width (HBW) and hardened depth (HD) of hardened bead profile respectively. These Figures indicate that the developed models are adequate because the residuals in prediction of each response are minimum, since the residuals tend to be close to the diagonal line. Furthermore, to verify the adequacy of the developed models, five confirmation experiments were carried out using new test conditions, but are within the experimental range defined early. Using the point prediction option in the software, the HI and HBW of the validation experiments were predicted using the previous developed models. Table 6 summarizes the experiments condition, the actual experimental values, the predicted values, error and the percentages of error.

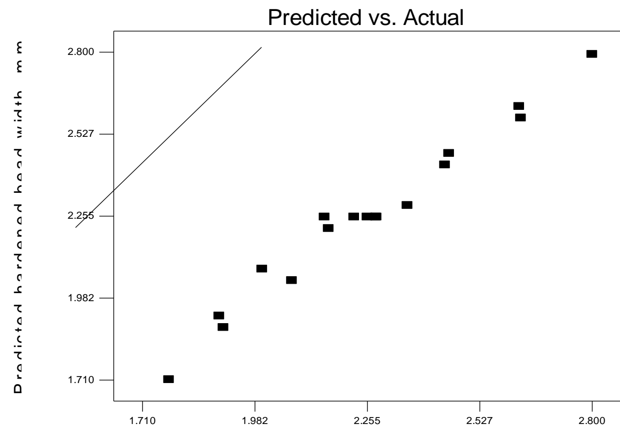

Figure 4. Scatter diagram of hardened bead width (HBW)

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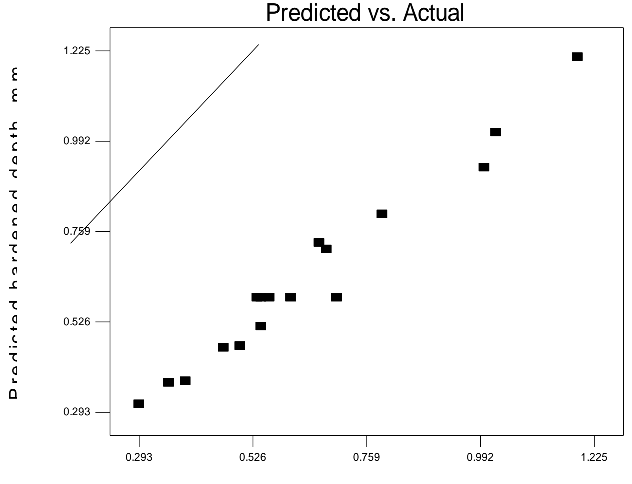

Figure 5. Scatter diagram of hardened depth (HD)

C. Effect of Process Factors on Hardened-Bead Parameters

C.1. Hardened bead width (HBW)

Figs 6-11 show the effect of process parameters on the hardened bead width (HBW). From the results it is clear that the two parameters laser power (LP) and scanning speed (SS) are significantly affecting the hardened bead width (HBW) as compared to focused position (FP). From the Figs. 6 and 7, it is evident that the hardened bead width linearly increases with increasing LP and decreasing SS. At lower beam travel speed the time available for the laser beam to direct contact with the surface is more and hence hardened bead width increases. Therefore the heat input decreases leading to the less volume of the base being melted, consequently the width of the hardened zone decreases. From the Figs. 8 and 9 it is clear that as LP increases and FP decreases the hardened bead width (HBW) increases Moreover, increase in defocused beam, or decrease in focused position i.e. from -10 mm, -20 mm, and -30 mm respectively means wide laser beam results in spreading the laser power onto wide area. Therefore, wide area of the base metal will melt leading to an increase in HBW or vise-versa. From the Figs. 10 and 11 it is observed that as the SS decreases and the FP decreases (i.e. from -10 mm to -30 mm) the hardened bead width increases.

The results show also that laser power (LP) plays very important role in the hardened bead dimensions. An increase in LP results in increase the HBW, because of increase in the power density.

C.2. Hardened depth (HD)

From the results it is studied that the parameters those significantly affecting the hardened depth are LP and SS. Effect of focused position on hardened depth is significant bur it has less influence as compared to LP and SS. These effects are due to following reasons: the increase in LP leads to an increase in the heat input, therefore, more molten metal and consequently more HD will be achieved. However, the idea is reversed in case of SS effect, because the SS matches an opposite with heat input (HI). From the Figs 12 and 13 it is seen that HD increases as LP increases and SS decreases. It is very important to note that in case of laser transformation hardening process main aim is to harden the surface with desired optimum depth. As much as possible instead of focusing the beam it is convenient to have defocused beam with negative focal length (i.e. from -10 mm,-20 mm and -30 mm), hence there is no loss of heat energy of laser beam above the focal point, since the laser beam is of converging type. Therefore laser heat input with minimum loss will be converged and concentrated on a specified localized area with desired hardened bead width and depth without spreading of laser power is achieved. Below the focal point or focused beam, the laser beam is of divergent type, results in spreading of laser power with maximum loss of heat input energy. Using a focused beam results in increasing the power density, which mean the heat will localize in small portion results in increasing in power density leading to better hardened bead width and depth which is desirable for laser transformation hardening (LTH). Therefore, due to the above reasons mentioned, it may be noted that to achieve the desired optimum hardened width and depth it is most convenient to have defocused laser beam with negative focused position (i.e. above the focal point) for example -10 mm, -20 mm and -30 mm.

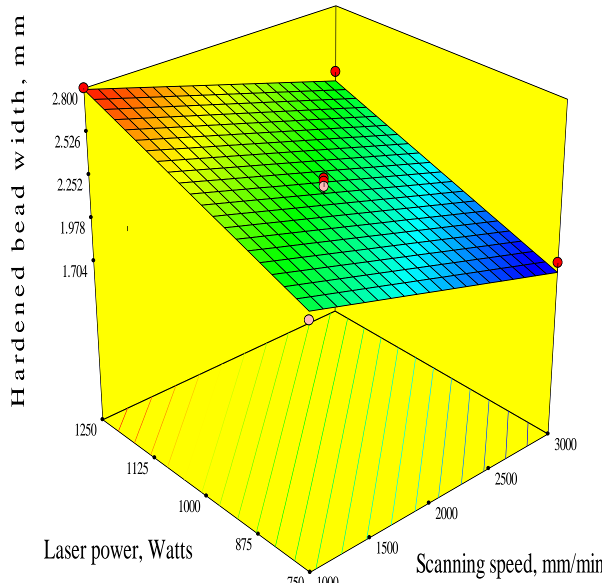

Figure 6. 3Dgraph shows the effect of LP and SS on the hardened bead width

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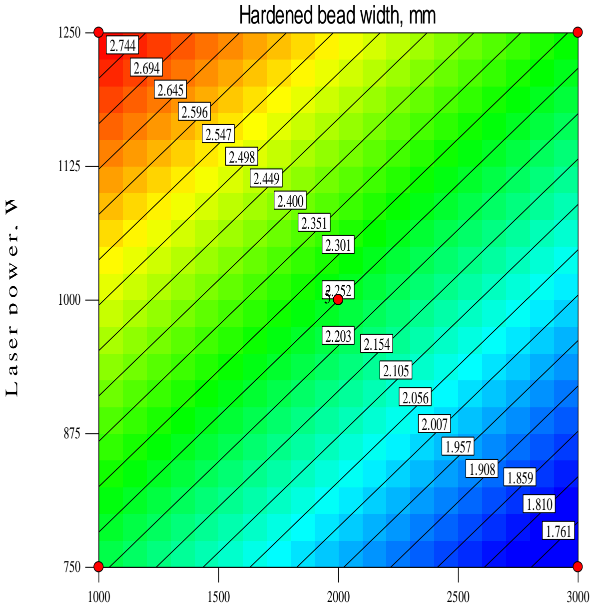

Figure 7. Contours graph shows the effect of LP and SS on the hardened bead width

From the Figs. 12 and 13 it is clear that hardened depth (HD) increases with increase in LP and decrease in SS. From the Figs.14 and 15, as LP decreases and defocusing increases (i.e FP from -10 to -30 mm) the HD decreases. It is also observed from the Figs.16 and 17, it is evident that as SS increases, hardened depth (HD) decreases considerably and as FP increases hardened depth (HD) decreases marginally. From the results obtained in Table 4 and from Figs.12-17, it is important to note that there is no large variation in the data of hardened depth (HD). Referring the Table 4 the range of HD lies between 0.293-1.191 mm and is of 0.898 mm variation only.

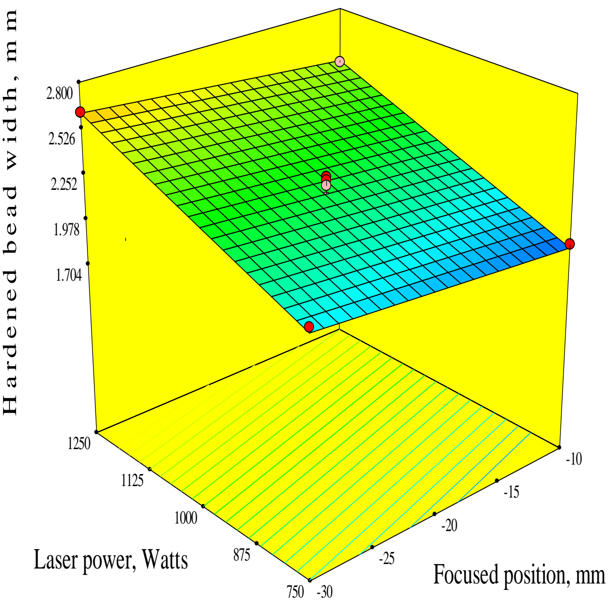

Figure 8. 3Dgraph shows the effect of LP and FP on the hardened bead width

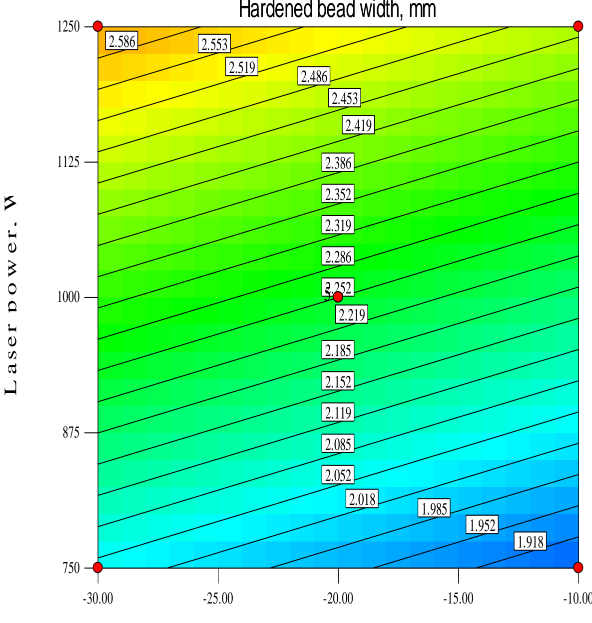

Figure 9. Contours graph shows the effect of LP and FP on the hardened bead width

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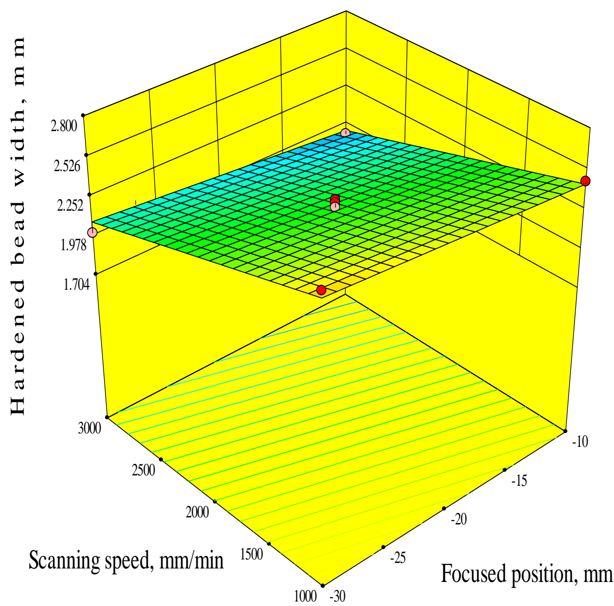

Figure 10. 3Dgraph shows the effect of SS and FP on the hardened bead width

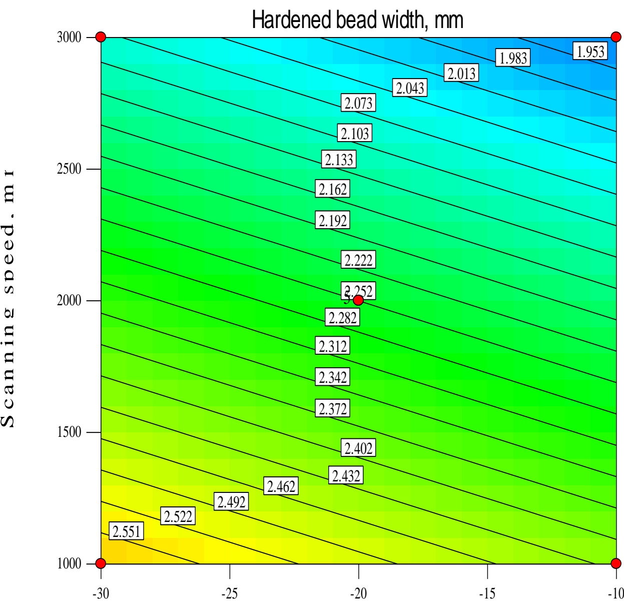

Figure 11. Contours graph shows the effect of SS and FP on the hardened bead width

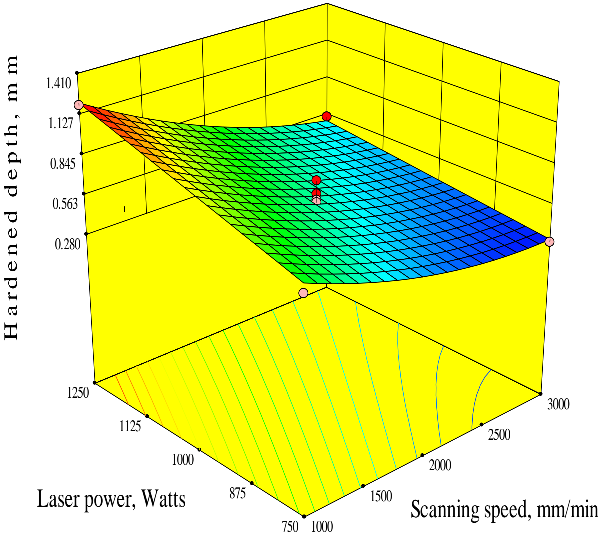

Figure 12. 3Dgraph shows the effect of LP and SS on the hardened depth

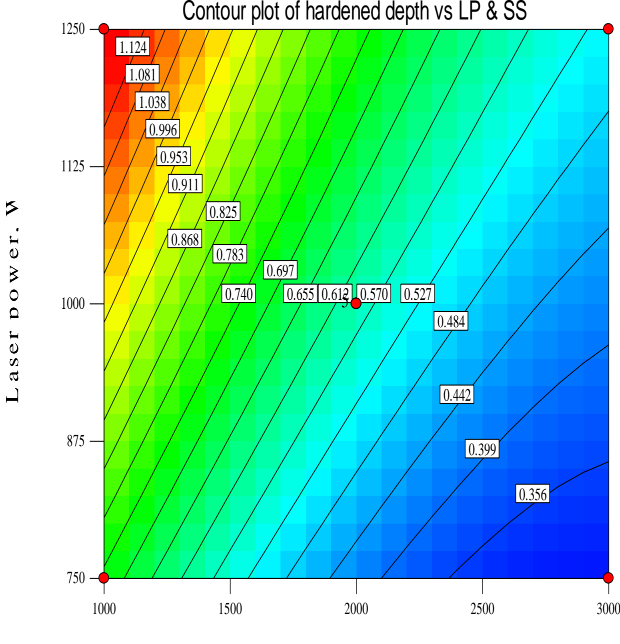

Figure 13. Contours graph shows the effect of LP and SS on the hardened depth

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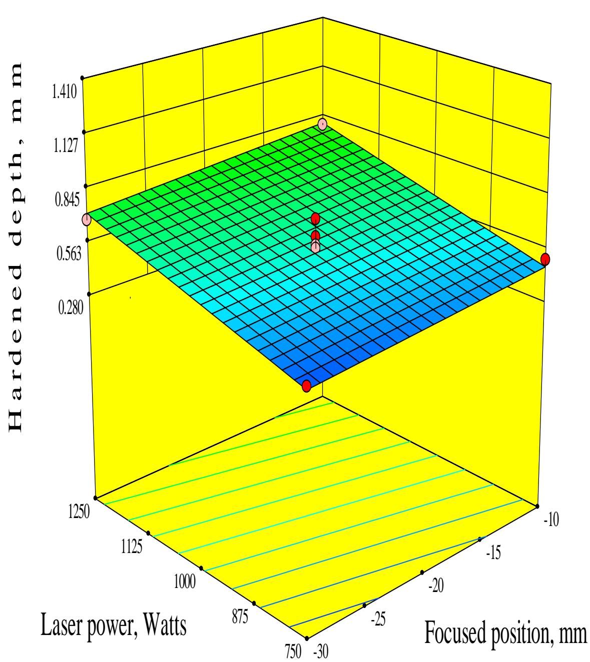


Figure 14. 3Dgraph shows the effect of LP and FP on the hardened depth

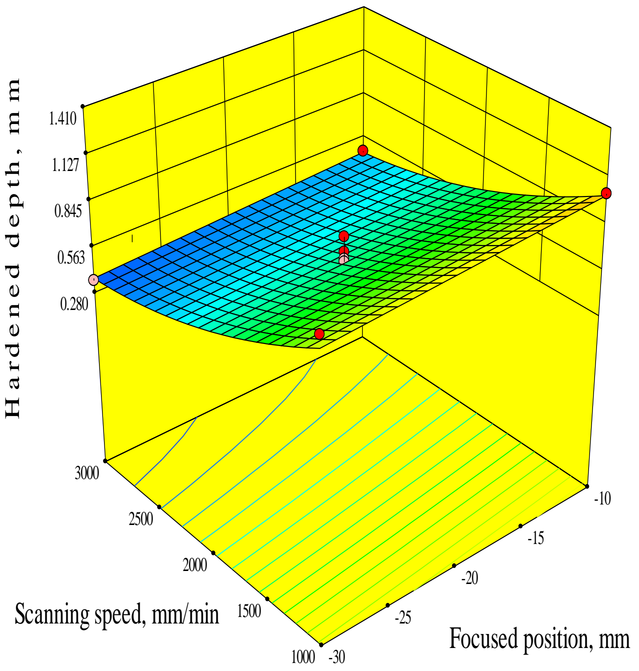
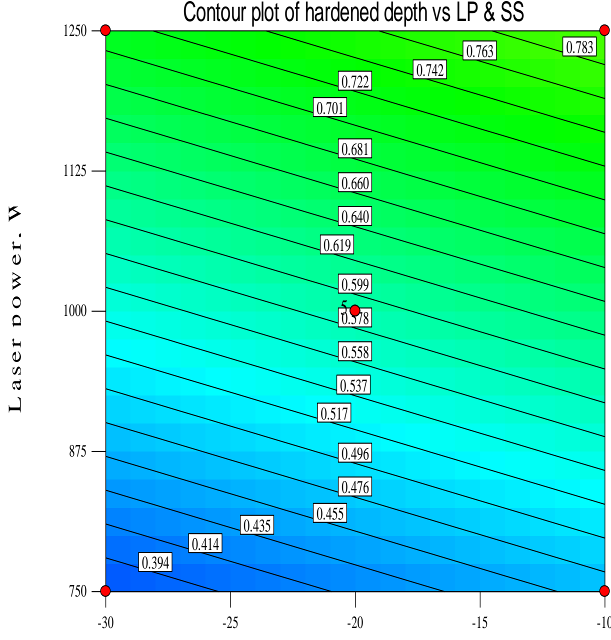
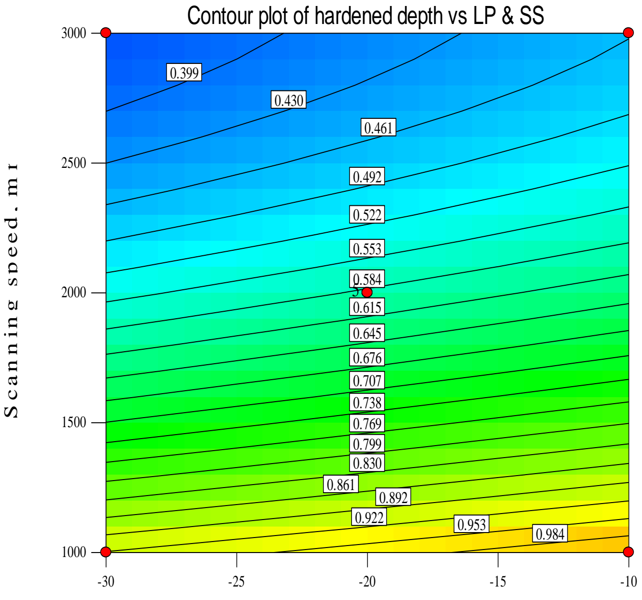


Figure 16. 3Dgraph shows the effect of SS and FP on the hardened depth



Focused position, mm

Figure 15. Contours graph shows the effect of LP and FP on the hardened depth



Focused position, mm

Figure 17. Contours graph shows the effect of SS and FP on the hardened depth

V. CONCLUSIONS

The following conclusions were drawn from this investigation within the factors limits considered. This paper has described the use of Design of Experiments (DoE) for conducting the experiments. Four models were developed

for predicting the heat input (HI), hardened bead width (HBW), hardened depth(HD), and angle of entry of hardened bead profile (AEHB) of the laser transformation hardened unalloyed titanium using response surface methodology(RSM). The following conclusions were drawn from this investigation within the factors limits considered.

1. Box-Behnken design can be employed to develop mathematical models for predicting laser hardened-bead geometry.
2. The desired hardened depth and width with high quality of laser transformation hardening (LTH) can be achieved by choosing the working condition using the developed models.
3. It is investigated that, in case of laser transformation hardening (LTH) though, as scanning speed increases depth of hardening decreases and vice-versa, but we are concentrating on desired optimum minimum depth. Therefore, both scanning speed and laser power have positive effect on all the responses investigated.
4. Bead width as well as depth of hardening linearly decreases with increasing scanning speed.
5. It is evident that the bead geometry provides a useful tool to manipulate the hardened bead width and hardened depth during LTH. It is clearly observed that the hardened width linearly increases defocused beam i.e. with higher beam spot size. Depth of hardened surface increases linearly with decrease in defocused position from -30 mm to -10 mm.

VI. ACKNOWLEDGEMENTS

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STATIC ANALYSIS AND WEIGHT OPTIMIZATION OF CRANKSHAFT IN SINGLE CYLINDER FOUR STROKE DIESEL ENGINE

Srinath Ingale[1], Dr. D. S. Badkar[2], Kailas Pathade[3] Govinda Patil[4] Sunil Rendale[5] Dayanand Patil[6]

[1,3]Assistant Professor of Mechanical Department, Dr. A. D. Shinde Collage of Engineering, Gadhinglaj-416502, Maharashtra, India
[2]Principal, Dr. A. D. Shinde Collage of Engineering, Gadhinglaj-416502, Maharashtra, India
[4,5,6] Students of Mechanical Department, Dr. A. D. Shinde Collage of Engineering, Gadhinglaj-416502, Maharashtra, India

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Abstract – *Reducing Weight is main problem in automobile industries. Since, if the vehicle weight is more the consumption of fuel is also more. And also if vehicle weight rises the cost also rises which indeed a main problem while buying an vehicle. The objective of the present work is to design and analyze the crankshaft with the material it is manufactured and also for the other metal alloys viz., EN9, SG700/2, SAE-1137.The solid model of the crankshaft was created in Solid Works. Model was imported in Altair Hyperworks for analysis by applying the normal load conditions. The model was tested for stress and deformation as the design constraints and also its frequency by performing modal analysis. After analysis a comparison is made between results of above materials mentioned in terms of deflections, stresses and frequency, to choose the best one.*

Key Words: **Crankshaft, EN9, SG700/2, SAE-1137, Solid Works, Hyperwork.**

1.INTRODUCTION

The main work of the crankshaft is to transform the translational mechanical work of the piston being carried forward and backward by the pneumatic work due to pressure change as a outcome of the combustion response. Crankshafts are mass volume manufacturing engine part and they are widely used in automobile engine. In an internal combustion engine, the pistons linear reciprocating motion is transformed into rotary motion using the crankshaft. There are lots of further uses of a crankshaft which covers from tiny single cylinder engines to big size multi cylinder marine crankshafts. The part connects the piston to the crankshaft via the connector pin used for the transfer of this energy; the connector pin energy to the tiny part of crankshaft axel that is offset comes from the main axis results in the rotation about the primary crankshaft axis. The crankshaft is also attached to the pull-start by the pull-start connection cup. When the pull-start chord is pulled, the energy is transferred to rotational energy of the crankshaft.

The crankshaft has transform the translational mechanical work of the piston to rotational mechanical work, its upcoming function is to convert this work to the driver pulley of the pulley-belt system. This is a important transfer of work due to its belt-pulley system that eventually replace this rotational mechanical work to the auger, causing it to rotate and collect the snow and additional material that is added by the auger. The movement that is related with the crankshaft is just this energy conversion. The crankshaft is located directly adjacent to the two-cycle gas engine, since it is attached to the piston by the connector pin.[5] This location further to the engine is a hot atmosphere that is caused by the convection of thermal energy off of the engine block's heat sink. The high temperatures in this atmosphere are cause for consideration when select the material for the crankshaft, which will be considered in the following section, with the help of geometry and appearance of the component.

2. GEOMETRIC MODELLING

To carry out CAE analysis of any component, the solid model of the same is essential. It is also called body in white. The CAD diagram shown in Fig. 1

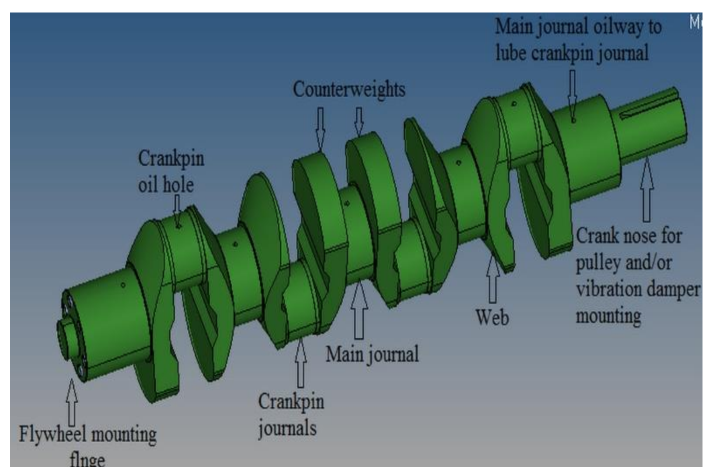

Figure 1- Crankshaft

2.1 MESHING

The creation of the FE-model initiates by importing the CAD model of the crankshaft from SOLID WORK to

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Hypermesh. The assembled SOLID WORK model is exported to Hypermesh in .igs format. Meshing of CAD model is done by using solid elements. Whole crankshaft is meshed using solid element tetra4 with an average element size of 5mm.

Each node has displacement components and rotational degrees of freedom. Quality checks are made in order to eliminate coincident nodes and coincident elements. A standard mesh sensitivity analysis is carried out in order to confirm that the results achieved are effectively not altering to the size of the elements used. Figure 2 shows the complete meshed model of crankshaft.

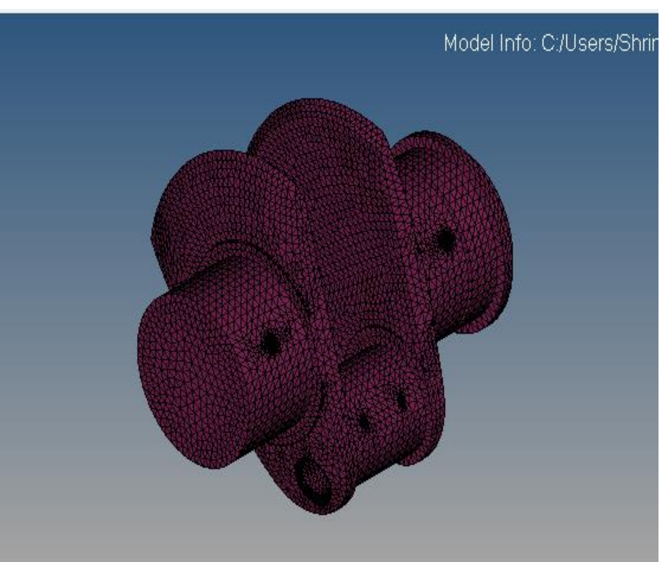

Figure 2: Meshed model of Crankshaft

Table 1: Types and number of elements

Total No. of Nodes	23305
Total No. of Elements	105613
Tria3 Elements	--------
Quad4 Elements	--------
Tetra4 Elements	105613
Hexa8 Elements	-------

2.2 MATERIALS AND PROPERTIES

Material properties need to be selected from standard material handbook. Most of the crankshafts are generally made of steel. Also the different materials with different compositions can be used for crankshafts which are shown in below table 2.

Table 2: Materials and properties

Material Description	Young's Modulus (E) MPa	Yield Strength (s) MPa	Poisson's ratio	Density ton/mm3
EN9	2.06e5	355	0.28	7.89e-9
SG700/2	1.72e5	471	0.28	7.15e-9
SAE-1137	2e5	435	0.285	7.8e-9

2.3 BOUNDARY CONDITIONS

The boundary condition is the applying of a load and/or constraint. In Hypermesh, boundary conditions are saved within a collector called load collectors. The function of the boundary conditions is to create and define constraints and loads on finite element models.

Constrained positions are showed in below figure 8.2.The load application is the major part in the analysis of a component. There are several kinds of loads like Point Load, Uniformly Varying Load and Uniformly Distributed Load.

The present crankshaft carries the UDL throughout its crankpin length. Where the total applied load is 39485.92N which is distributed along the length of the crankpin as well as the crankshaft is constrained at the both ends. Length of the pin is 40 mm. The total load and constrained applied on the crankshaft is as shown in below figure 3.

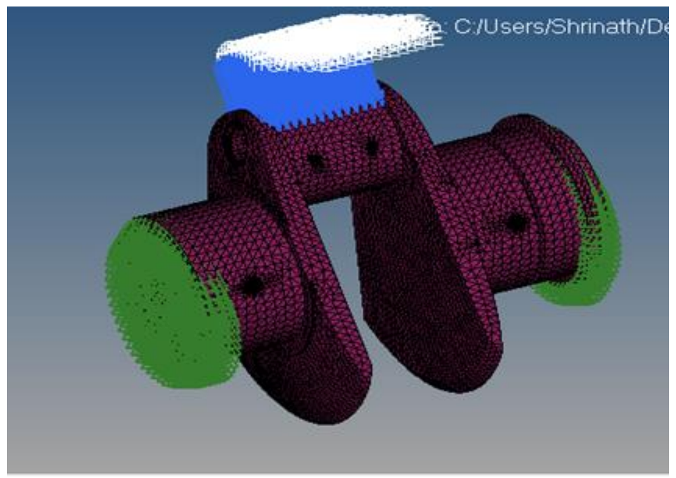

Figure 3: Boundary conditions.

3. SIMULATION AND RESULTS

Simulation is the processes of product validation were the product is tested with defined boundary conditions and assumed parameters. The process of simulating anything starts with model development; this model indicates the main function, characteristics or behaviors of the chosen physical process. The model represents the system itself, whereas the simulation represents the operation of the system over time.

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In this paper we are taken a crankshaft and by changing its material properties the simulations are carried out. And finally the results of the simulations were compared.

3.1 EN9

For EN9 material, static analysis results are maximum displacement is 0.02128mm and maximum von-misses stress is 146.7Mpa. For free-free modal analysis frequency is 8378.6Hz and for constrained frame frequency is 6281.8Hz. The result images are shown in figure 4, 5, 6 and 7.

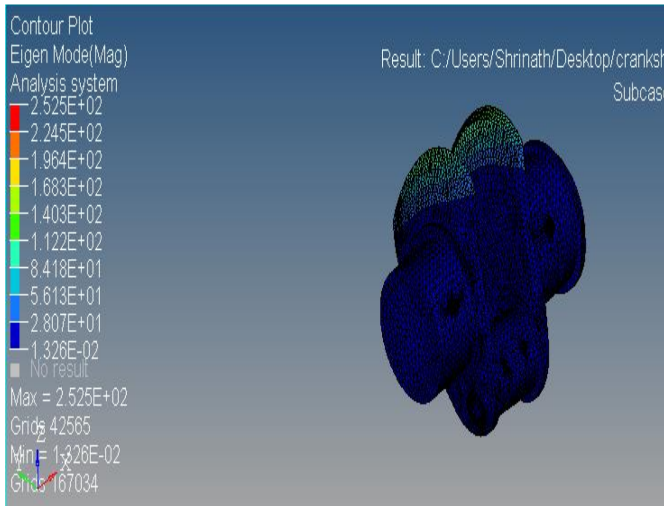

Figure 4: Free-Free Modal Analysis For EN9

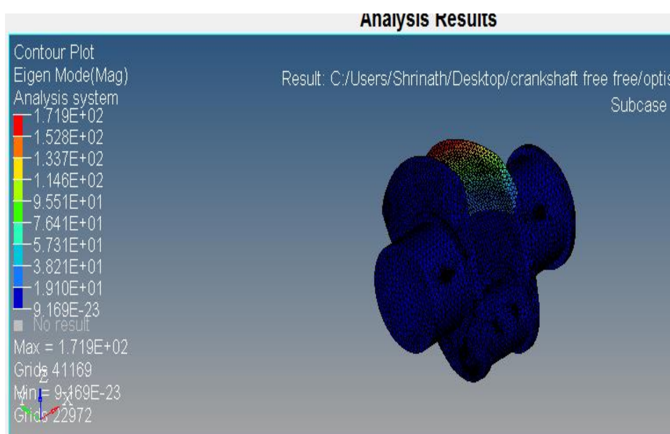

Figure 5: Constrained Modal Analysis For EN9

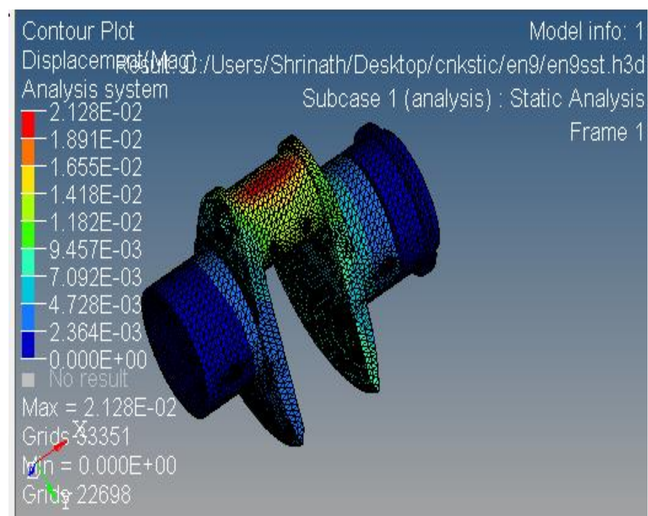

Figure 6: Displacement For EN9

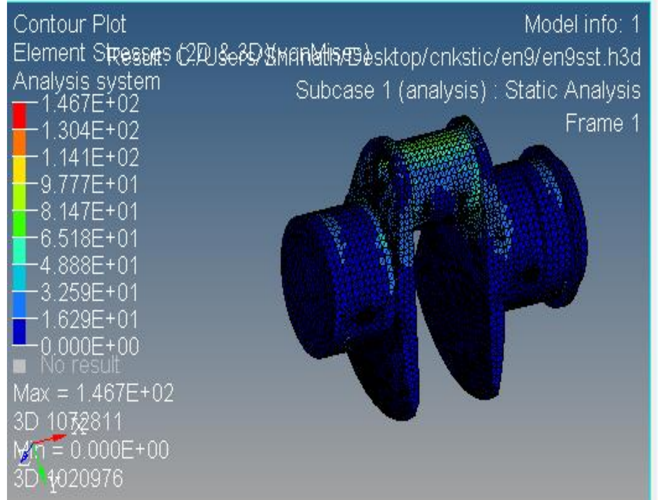

Figure 7: Von-Misses Stress For EN9

3.2 SAE-1137

For SAE-1137, static analysis results are maximum displacement is 0.02188mm And maximum von-misses stress is 146.1Mpa.For free-free modal analysis frequency is 8321.3Hzand for constrained frame frequency is 6239.4Hz. The result images are shown in figure 8,9,10 and 11.

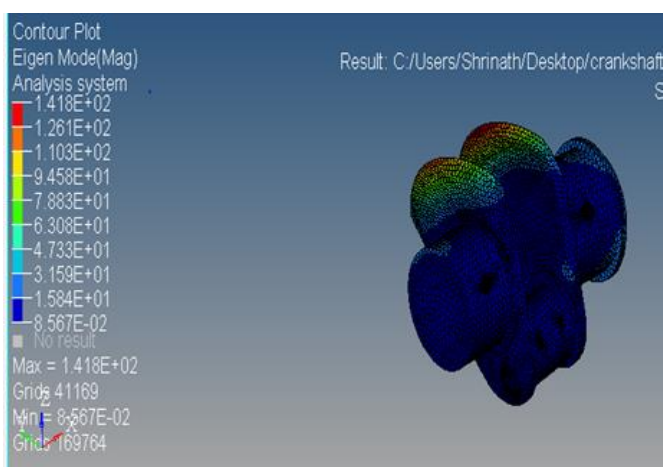

Figure 8: Free-Free Modal Analysis For SAE-1137

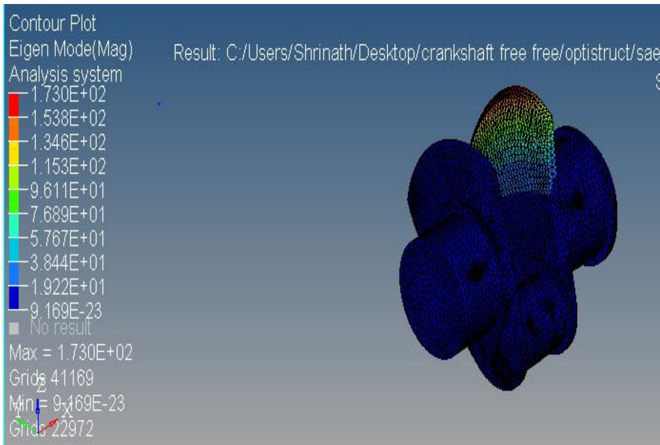

Figure 9: Constrained Modal Analysis For SAE-1137

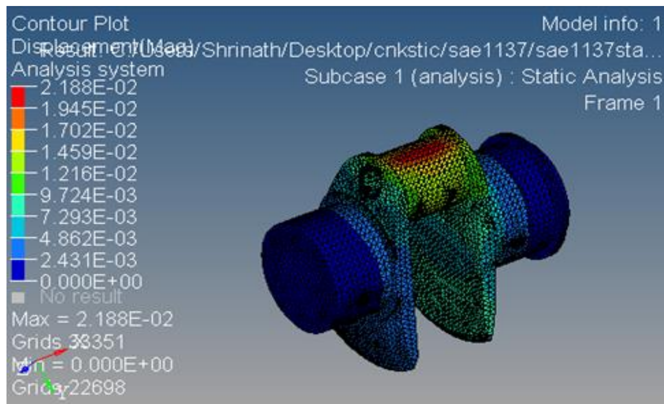

Figure 10: Displacement SAE-1137

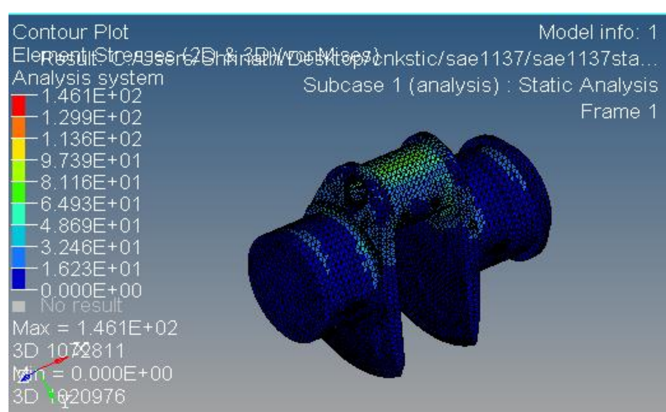

Figure 11: Von-Misses Stress SAE-1137

3.3 SG700/2

For Low alloy steel SG700/2, static analysis results are maximum displacement is 0.0254mm and maximum von-misses stress is 146.7Mpa. For free-free modal analysis frequency is 8023.7Hz and for constrained frame frequency is 6014.6Hz. The result images are shown in figure 12, 13, 14, and 15.

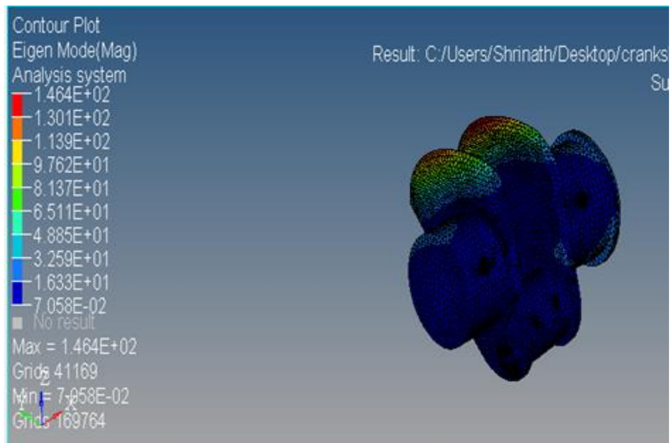

Figure 12: Free-Free Modal Analysis For SG700/2

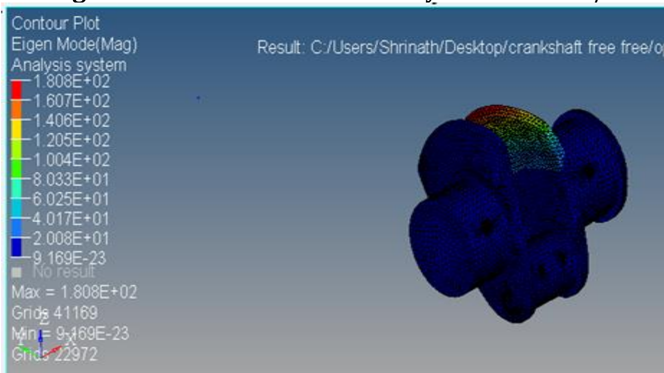

Figure 13: Constrained Modal Analysis For SG700/2

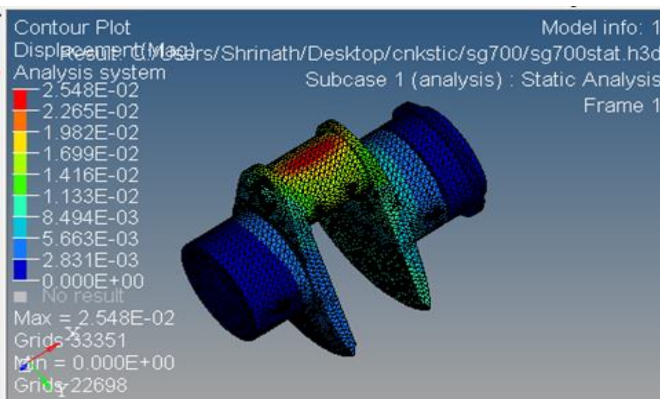

Figure 14: Displacement For SG700/2

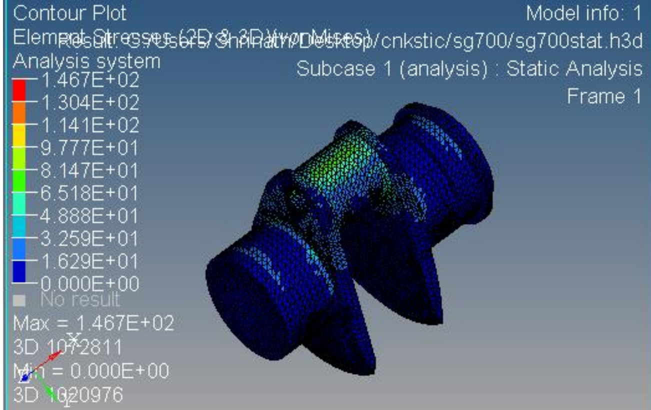

Figure 15: Von-Misses Stress For SG700/2

4. SUMMARY OF RESULTS

In this section we will discuss and compare the results of above simulations. The table 8.1 shows the summary of simulation results. The table shows the comparison between the simulation results of ladder chassis frame with different materials.

Table 3: Summary of Results

Materials	Static Analysis		Modal Analysis		Weight (kg)	Price (INR/kg)
	Displacement (mm)	Stress (MPa)	Free-free Frequency (Hz)	Constrained Frequency (Hz)		
EN9	0.02128	146.7	8378.6	6281.8	2.826	
SG700/2	0.0254	146.7	8023.6	6014.6	2.5609	43.15
SAE-1137	0.02188	146.1	8321.3	6239.4	2.794	47.45

5. CONCLUSIONS

In this project work, simulation is carried out on crankshaft with different materials. Here Altair Hypermeshv11.0 tool is used for meshing the complete model and passed the quality criteria's such as Quality Index, Skewness, Warpage, Jacobian etc. The FE model is successfully analyzed using RADIOSS and OPTISTRUCT software. After completing the simulations displacement, stress and frequency results are compared. From the comparison of simulation results we can concluded that:

1. The displacement is less i.e. 0.02128mm in EN9 material compared to other materials.
2. The von misses stresses are found almost same in all the three materials (EN9, SG700/2, SAE-1137) i.e. 146.7MPa under given boundary conditions.
3. The crankshaft made of SG700/2 material has less weight of 2.5609kg compared to other materials analyzed.
4. 10% weight reduction is observed by using SG700/2 with respect to EN9.
5. As compared to the cost of the SG700/2 is having the lower with compared to other material.
6. The frequency seen for material SG700/2 in free-free analysis is 8023.6Hz which is relatively lower and comparable with other materials.
7. The frequency seen for material SG700/2 in constrained analysis is 6014.6Hz which is relatively lower and comparable with other materials.
8. The percentage of weight reduction is 10% in single cylinder engines where as it would be even lower for four cylinder engine crankshaft.

ACKNOWLEDGEMENT

It gives us an immense pleasure to write an acknowledgement to this Paper, a contribution of all people who helped us realize it.

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BIOGRAPHIES

Prof. Srinath Ingale
Working as a assistant professor in Mechanical Department, Dr. A. D. Shinde College of Engineering, Bhadgaon.

Dr. D. S. Badkar
Dr. D. S. Badkar working as Principal in Dr. A. D. Shinde College of Engineering, Bhadgaon. PhD from NIT Bhopal.

Prof. Kailas S. Pathade
Working as Head of Department in Mechanical Department, Dr. A. D. Shinde College of Engineering, Bhadgaon.

Mr. Govinda Patil
Last year student of Mechanical Department, Dr. A. D. Shinde College of Engineering, Bhadgaon.

Mr. Sunil Rendale
Last year student of Mechanical Department, Dr. A. D. Shinde College of Engineering, Bhadgaon.

Mr. Dayanand Patil
Last year student of Mechanical Department, Dr. A. D. Shinde College of Engineering, Bhadgaon.

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Design and Implementation of Track Following & Obstacles Avoiding Robotics System Based on Programmable Technique

Asif Mohammad Shikalgar,Suraj Shankar Patil ,Pallavi Machindra Pawar,Pooja Khashaba Mane, Sonali Sunil More, Neha Sunil Kshirsagar

Dept. of Electronics & Tele-communication Enginnering, YSPM's YTC SATARA ,India

Abstract

Line following robot detects a visual line on a smooth surface area embedded on the floor and follows it. The path line is predefined and visible with a black line on the white surface area or the opposite. A line follower robot is designed, developed and implemented on a path of black on white or white on black using IR sensor and ultrasonic for object detector. The Arduino coding is developed on using C programming and tested and verified. The proposed system can be implemented on any commercial, industrial, medical and also in educational labs.

Keywords: Line follower, Obstacle detection, IR sensor and Ultrasonic sensor.

1 Introduction

The line following robot is the self-operating intelligent machine that follows a drawn line on a floor area and the path line can be visible as black on a white surface area; or a white line on the black surface area. It is an autonomous robot which identify and tracks either on a black line in white surface area or a white line in black surface area. Line following robot must be able to detect a specified line and maintain track on it and do the assign jobs. For performing job, the given path line must be followed by the designed and developed robot for special situations. The developed system composed of input, process and output parts. First read the black/white or white/black path on considered floor and take input signal for transmission into microcontroller (Arduino UNO) in a process that can be asked and made the decisions. Microcontroller decided based on the received inputs that can change (if needed) to be made directions or speeds of the robot. It converts the result to any directions which can be sent to the line follower speed. The system sends the first or previous adjusted control signals to speed and directions of line follower robot.

To design a line follower an ultrasonic sensor is needed, which is a device that can measure distance to an object by using sound waves. It computes the distance between the object and the line follower by sending a sound wave at a specific frequency and listening for that sound wave to bounce back. It is important to understand that some objects might not be detect by ultrasonic sensor. This can be applied for military purposes, delivery services, transportation systems, blind assistive applications. Finally, there are many annual line follower robot's competitions organized by universities or industries around the world.

2 LINE FOLLOWING PRINCIPLE

Here in this line follower robot we are using two IR sensor modules left sensor and right sensor. When both left and right sensor senses white then robot move forward (Fig. 1.1). If left sensor comes on black line, then the robot turns left side in the black line. (Fig. 1.2). If right sensor sense black line, then the robot turn right side until both sensors at white surface. When white surface comes robot starts moving on forward again (Fig. 1.3). If both sensors come on black line, robot stops (Fig. 1.4).

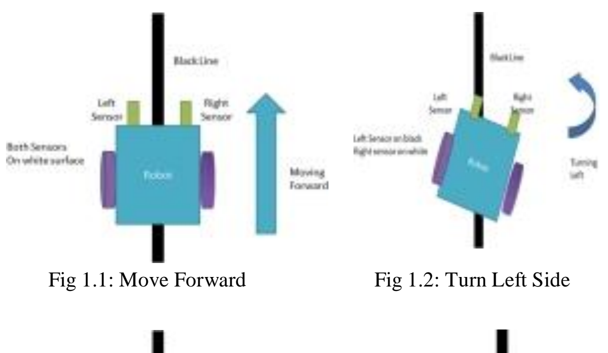

Fig 1.1: Move Forward

Fig 1.2: Turn Left Side

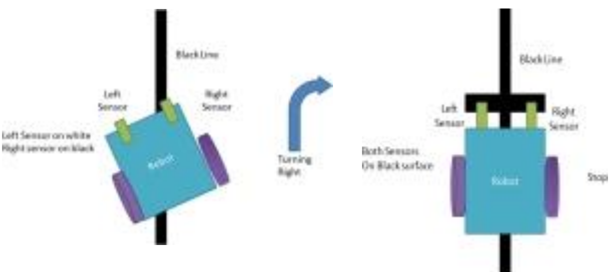

Fig 1.3: Turn Right Side

Fig 1.4: Stop

Fig. 1: Principle of Line Follower

3 PATHS OF LINE FOLLOWER

Line follower robot follows path drawn on the floor. The line will be mainly black on a white surface. If it occurs any line break on its way, the robot will go forward. If it finds a cross line, the robot will stop. Lines and robot movements can be changed by using programming code easily. Some lines are that the robot can follow:

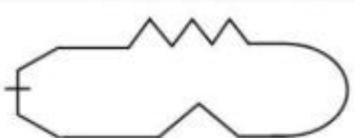

Fig. 2: Sample Path

The robot will follow a bad angle of 45° and cycle or bad curves. It will stop when it finds a cross black line.

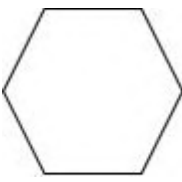

Fig. 3: Polygon Shape Path

On any kind of polygon, it can follow the line and maintain a particular speed.

Fig. 4: Cycle and Hard Curve

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The line may have cycles and unwanted curves that it has to follow on narrow space or moving one room to another.

4 EXPERIMENTAL EQUIPMENT

The proposed robot is made of several components and they are: Arduino Uno R3, Arduino IDE, Ultrasonic Sensor (HC-SR04), Sonar Sensor Base, Digital IR sensor array, Motor Driver (L298N), Battery (900mAh), Two DC motors with Chassis Board, Mini Bread Board and Jumper Wires and are presented in Fig. 5.

Fig. 5: Necessary Equipment

5. CIRCUIT DIAGRAM AND CODE

A. *Circuit Diagram*

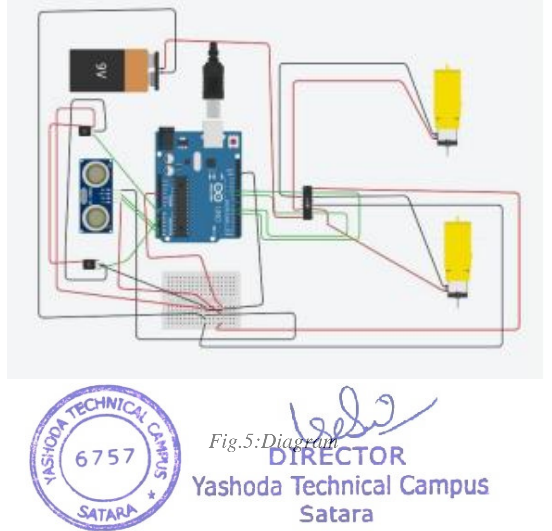

Fig.5:Diagram

A. PinConnection

Table1:Connectionbetween MotorDriverandArduino

Motor Driver	Arduino UNO
IN1 IN2 IN3 IN4VCC/ 12VGND 5V	10 9 6 5 Vin/5v GND5 V

Table1

Table 2: Connection between IRSensorandArduino

IRSensor	ArduinoUNO
Sensor 1:VCCG NDOUT Sensor 2:VCCG NDOUT	VCC GND A0 VCC GND A1

Table2

Table 3: Connection between Ultrasonic Sensorand Arduino

Ultrasonic Sensor	ArduinoUNO
GND ECHO TRIG VCC	GND A3 A5V CC

Table3

B. SourceCode

The complete code of the of the proposed system ispresented below

.

```
#define trigpin A5#defineechopinA4intmotor_l1=6;intmotor_l2=5;int motor_r1 =
10;int motor_r2 = 9;intspeed=115;
intfrontdist;long duration;intsetdist = 10;int L_S = A0;int R_S = A1;void
setup(){
pinMode(motor_l1,OUTPUT);pinMode(motor_l2,OUTPUT);pinMode(motor_r1,OUTPUT);pin
Mode(motor_r2,OUTPUT);pinMode(trigpin,OUTPUT);pinMode(echopin,OUTPUT);pinMode(
L_S,INPUT);pinMode(R_S,INPUT);Serial.begin(9600);delay(1000);
}
void loop(){frontdist=data();
Serial.println(frontdist);if(frontdist>setdist){
if((digitalRead(L_S) == 0) && (digitalRead(R_S) ==0){forword();}
if((digitalRead(L_S) == 0) && (digitalRead(R_S) ==1){turnRight();}
if((digitalRead(L_S) == 1) && (digitalRead(R_S) ==1){turnLeft();}
}else{turnLeft();delay(350);forword();delay(1000);turnRight();delay(200)
;forword();delay(500);
}
}
long data(){Longdata(){
digitalWrite(trigpin,LOW);delayMicroseconds(2);digitalWrite(trigpin,HIGH);delayMicrosecon
ds(10);duration=pulseIn(echopin,HIGH);returnduaration/ 29/2
}
Void stop(){analogWrite(motor_11,0);
analogWrite(motor_l2,0);
analogWrite(motor_r1,0);
analogWrite(motor_r2,0);
}
Void forward(){analogWrite(motor_11, speed);analogWrite(motor_l2,0);
analogWrite(motor_r1, 0);analogWrite(motor_r2,speed);
}
Void backword(){analogWrite(motor_11, 0);analogWrite(motor_l2,
speed);analogWrite(motor_r1,speed);analogWrite(motor_r2,0);
}
Void turnRight(){analogWrite(motor_11, 0);analogWrite(motor_l2,
speed;analogWrite(motor_r1, 0);analogWrite(motor_r2,speed);
}
Void turnLeft(){analogWrite(motor_11, speed);analogWrite(motor_l2,
0);analogWrite(motor_r1, speed);analogWrite(motor_r2,0);
}
```

6.APPLICATIONS

There are some identified application of the designed line follower robot and they are presented below:

(a)It can be used to deliver mail within an office building, industrial floor, medical ward and any robotics lab for education.

(b)It can be any mass transit system either bus stations of any airports.

(c)Line follower robots can be applied in military spy kids moving activities.

7.CONCLUSION

A line following robot is designed, developed and implemented that does not need any remote controller, Bluetooth, Wi-Fi, GSM, etc. This will run automatically with following a given line using Arduino microcontroller. This line follower robot is low cost but very effective for various purposes.

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This approach can be applied in different sectors like an office building, industrial floor, medical ward and any robotics lab for education purposes.

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Fingerprint and IOT Based Exam Hall Authentication

[1]*Omkar Nikam,*[2]*Indrayani Kashid,*[3]*Ankita Bhosale,*[4]*Pooja Phadtare,*[5]*Komal Kale*

[1,2,3,4,5]*Department of Electronics and Telecommunication Engineering, Yashoda Technical Campus Wadhe,Satara,India*

Abstract

Security is crucial all around. Access into institutions, examination centers, organizations or even estates ought to be controlled and closely monitored through a verification system. The method of authenticating a student for an examination has an obvious problem such as presentation of fake clearance card, impersonation and so on and the unethical manner associated with the examination is a grim issue that requires the stakeholders in academic area to seek for alternative means of authenticating student for examination, because the manual paper-based clearance process is fundamentally flawed. Sequel to that, a dependable and effective system is designed to tackle the issues of the convectional technique. The system will verify the understudy using fingerprint biometrics technique and generate a report which can serve as an attendance.Multi biometric is an authentication technology using different biometric technology such as fingerprint, facial features and, vein pattern. The process for allowing student to sit for an examination in most universities has been through the presentation of medium of identification such as ID cards,, fees clearance card, photo cards, etc.

Keywords-Fingerprint Sensor, IOT module, authentication, Impersation.

1 INTRODUCTION

All academic institution have certain criteria for admitting students into examination hall. Hence accurate record of attendance and fees payments is necessary. To verify identity of person it is very critical and important task in society. Cash terminals, access control, examination pass identity; internet transactions are the basic examples of security issues where the identities of the users are important and useful.

Most of the universities adopted paper means authentication for eligibility of students for examination. This is issued by the university's examination and record units.

This contains vital information needed in identifying candidates. These may include the student's name, passport photographs and school's authentication stamps. This is known as 'examination pass'. It is the method devised by the institution's authorities in identifying eligible candidates for various examinations.

It is note that with the level of information provided, they still open to student as some of information displayed. By this pass can still be tampered with for the sole purpose of impersonations and other examination fraud as the case may be. Some of students get duplicate pass of examination hall that leads to cheating or fraud.

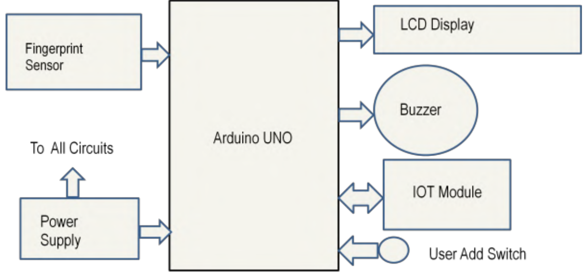
2 PROBLEM DEFINITION-

The problems which are encountered in the previous identification systems are:

- Student impersonation
- Insecure authentication of students
- Manual Verification of students
- Corruption in Examination System

3 METHDOLOGY-

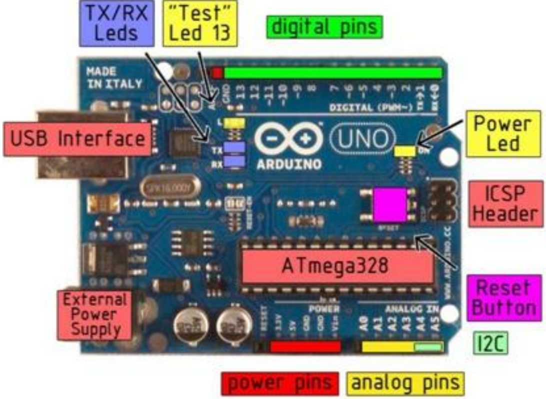
BLOCK DIAGRAM-



4 HARDWARE DESCRIPTION

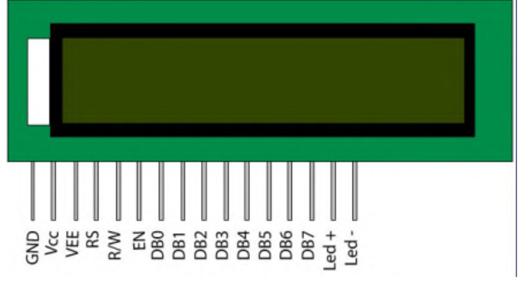
ARDUINO UNO-

The Arduino Uno is a microcontroller board based on the Arduino328 (datasheet). It has 14 digital input/output pins (of which 6 can be used as PWM outputs), 6 analog inputs, a 16 MHz crystal oscillator, a USB connection, a power jack, an ICSP header, and a reset button. It contains everything needed to support the microcontroller; simply connect it to a computer with a USB cable or power it with a AC-to-DC adapter or battery to get started. The Uno differs from all preceding boards in that it does not use the FTDI USB-to-serial driver chip. Instead, it features the Arduino8U2 programmed as a USB-to-serial converter. "Uno" means one in Italian and is named to mark the upcoming release of Arduino 1.0. The Uno and version 1.0 will be the reference versions of Arduino, moving forward



16X2 ALPHANUMERIC DISPLAY-

LCD (Liquid Crystal Display) screen is an electronic display module and find a wide range of applications. A 16x2 LCD display is very basic module and is very commonly used in various devices and circuits. These modules are preferred over seven segments and other multi segment LEDs.
A 16x2 LCD means it can display 16 characters per line and there are 2 such lines.



IOT MODULE-

The ESP8266 Wi-Fi Module is a self contained SOC with integrated TCP/IP protocol stack that can give any microcontroller access to your Wi-Fi network. The ESP8266 is capable of either hosting an application or offloading all Wi-Fi networking functions from another application processor.

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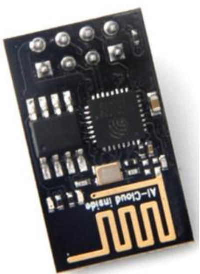

Fingerprint sensor-

The fingerprint algorithm extracts features from the acquired fingerprint image and represents the fingerprint information. The storage, comparison, and search of fingerprints are all done by operating fingerprint features. Fingerprint processing includes two processes: fingerprint registration process and fingerprint matching process.

Buzzer-

Piezoelectric Sounders / Buzzers are sound components prepared by incorporating a piezoelectric vibration plate in a plastic case (resonator)..
This characteristic allows them to be used in a wide range of applications. They come as the SMD type, which is optimal for small, high-density mounting and the pin type, which can be used for general purposes.

5 WORKING PRINCIPLE-

It is divided into two stages. They include the:
I. Enrollment
II. Verification

Enrollment:
The device comes on when it is powered. When an individual is to be registered, a number is assigned to him/her using the keypad. This number assigned is automatically stored in the EEPROM of the ARDUINO IC used. When the individual places his/her hand on the finger print module, it captures the print and transfers it into the EEPROM and later stores in the memory card. This process is repeated to be stored in the memory of ARDUINO IC for confirmation. All the activity performed by the microcontroller is being displayed on the liquid crystal display (LCD).
Verification:
The system verifies the student by scanning the fingerprint and compares with the ARDUINO captured image. If the image is registered, it prints out the individual's identification number to confirm his eligibility.
When a wrong finger is placed on the module, it scans the image, sends it to the ARDUINO. This browses through the images in its memory

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and if nothing is found, it prints out a message stating that the person in question has no personal details in its memory. A message "NOT REGISTERED" is displayed on the screen. Figure 1 and 2 depict the flowchart of the enrollment and verification stage.

6 RESULT-

Fingerprint matches

Fingerprint not matched

Sr No.	Condition	Result
1.	When fingerprint is matched with already stored data.	It displays the individual's identification number. And door of examination hall will open.
2.	When fingerprint is not matched with already stored data.	A message not matched is displayed on screen. And door of examination hall will not open.

ADVANTAGES-

- This project can be used to easily indetification of authorised person.
- Security
- Complexity of design can be reduced and made compact.
- Unique Identification of Individual Level

DISADVANTAGES-

- The sensors are costly.
- If power supply fails circuits won't work.

7 FUTURE SCOPE-

Further research can be carried out to have more than one biometric technique like a fingerprint and facial recognition and also in very large scale organization, that the memory card cannot contain all the data. A hard-drive can be used.

8 COCLUSION-

The system will successfully identify and verify the registered understudy fingerprint and stored the verified understudy so that the lecturer can retrieve the list of all understudies that was verified to take an examination. The system gives the time when the understudy was verified. In other words, the system generates a report in real time using the understudy fingerprint to avoid or prevent impersonation.

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IOT Based Underground Drainage and Manhole Monitoring System for Cities

Vishakha Bhojane, Anjali Bhosale, Ankita Sankpal, Shubhada Bhosale, Ujjwala Patil

[2]UG Students, Department of Electronics and Telecommunications, Satara College Of Engineering and Management System, Limb, Satara India

ABSTRACT

The Internet of Things (IoT) consists of real life objects, communication devices attached to sensor networks in order to provide communication and automated actions between real world and information world.IoT came into existence because, without human interaction computers were able to access data from objects and devices, but it was aimed at, to overcome the limiting factors of human entered data, and to achieve cost, accuracy and generality factors. Sensor Network is a key enabler for IoT paradigm. This paper represents the implementation and design function of an Underground Drainage and Manhole Monitoring System (UDMS) for IoT applications. The vital considerations of this design are low cost, low maintenance, fast deployment, and high number of sensors, long life-time and high quality of service. The proposed model provides a system of monitoring the water level and atmospheric temperature and pressure inside a manhole and to check whether a manhole lid is open. It also monitors underground installed electric power lines. In real time, UDMS can remotely monitor current states of the manholes.

Keywords: IOT, Manhole Monitoring, Underground Drainage and Manhole Monitoring System

1 INTRODUCTION

Many cities in India have an underground drainage system and Municipal Corporation manages the sewage system for clean and healthy climate. The water in the drainage system is sometimes mixed with pure water, due to poor maintenance. The drainage system can spread to the atmosphere and diseases that caused by pathogens. Drainage is disrupted over various seasons due to change in climate, and the environment is volatile and disturbs people and disturbs their daily lives. To solve all the problems of the drainage system and to inform the municipal corporation by sending Blynk notification of the state of the drainage system, so that the officials can take the necessary steps to repair drainage system. The gas itself formed inside the bio-waste drainage system was also detected using a gas sensor to prevent explosion by the pressure inside the drainage system. So our aim of this idea is to track the drainage system using the sensor. When the sewage system is blocked or wateroverflows or the drainage lid is removed, the drainage is monitored using sensor and sensed information is transmitted via Blynk to the nearby municipal corporation official using integrated Wi-Fi, and the water overflow and gas value is displayed live in the cloud for later analysis. And the particular drainage's GPS location is also sent via Blynk Server.

2 Objective

- The main objective of this project is to keep the city clean, safety and healthy.
- If the drainage maintenance is not proper it will create problem for routine life, traffic may get jammed infectious disease may get spread and there is a chance of occurrence of accidents
- The vital consideration of this design are low cost, low maintenance, fast development, and high number ofsensors, long life time and high quality of services.

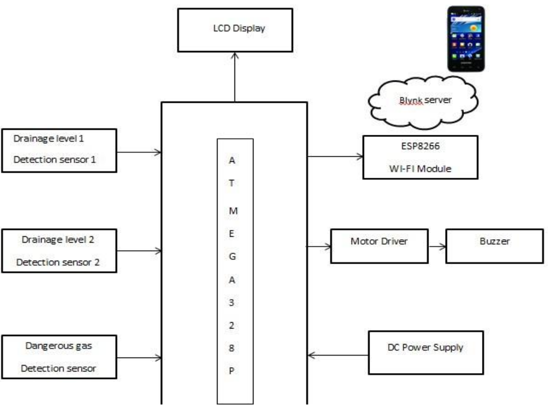
3 Motivation

If the drainage maintenance is not proper the pure water gets contaminate with drainage water and infection diseases may get spread. It will create problem for routine life. The traffic may get jammed, the environment becomes dirty and totally it upsets the public. If the manhole lid is not closed properly there is a chance of occurrence of accidents and also people or animals may get fall into the drainage. This problems is very interesting suppose imagine if we should have a remote monitoring system to monitor the internal states of the manhole and then we can solve this problem efficiently. These problems occur due to environmental changes like rainy season.

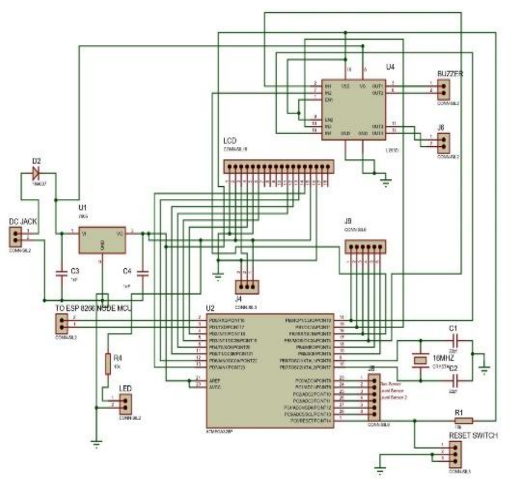
4 Proposed Work

ATmega328P Microcontroller receives live data collected from different sensors. The different sensors are Water level detection sensor and Gas sensors. The water level detection sensor and gas detection sensor sense the current states of the manholes. Sensors send sensed data to microcontroller. The output obtained from controller is sent to mobile device or computer using Blynk server. Blynk server is responsible for all interactions between smartphones and equipment.

5System Architecture



6 Circuit Diagram



Circuit Diagram Description

- In above the circuit diagram of IoT based underground drainage and manhole monitoring system for cities.
- In that we connect gas detection sensor, water level1 detection sensor1 and water level2 detection sensor2 to ATmega328p microcontroller.
- The microcontroller is connect to the ESP8266 wifi module.
- We get output on blynk application by blynk server.

7 ARDUINO IDE Software

8 Software Module

The Arduino Integrated Development Environment (IDE) is a cross-platform application (for Windows, macOS, Linux) that is written in functions from C and C++. It is used to write and upload programs to Arduino compatible boards, but also, with the help of third-party cores, other vendor development boards.

The source code for the IDE is released under the GNU General Public License, version 2. The Arduino IDE supports the languages C and C++ using special rules of code structuring. The Arduino IDE supplies a software library fromthe Wiring project, which provides many common input and output procedures. User-written code only requires two basic functions, for starting the sketch and the main program loop, thatare compiled and linked with a programstub main() into an executable cyclicexecutive program with the GNU toolchain, also included with the IDE distribution. The Arduino IDE employs the program avrdude to convert the executable code into a text file in hexadecimal encoding that is loaded into the Arduino board by a loader program in the board's firmware. By default, avrdude is used as the uploading tool to flash the user code onto official Arduino boards.

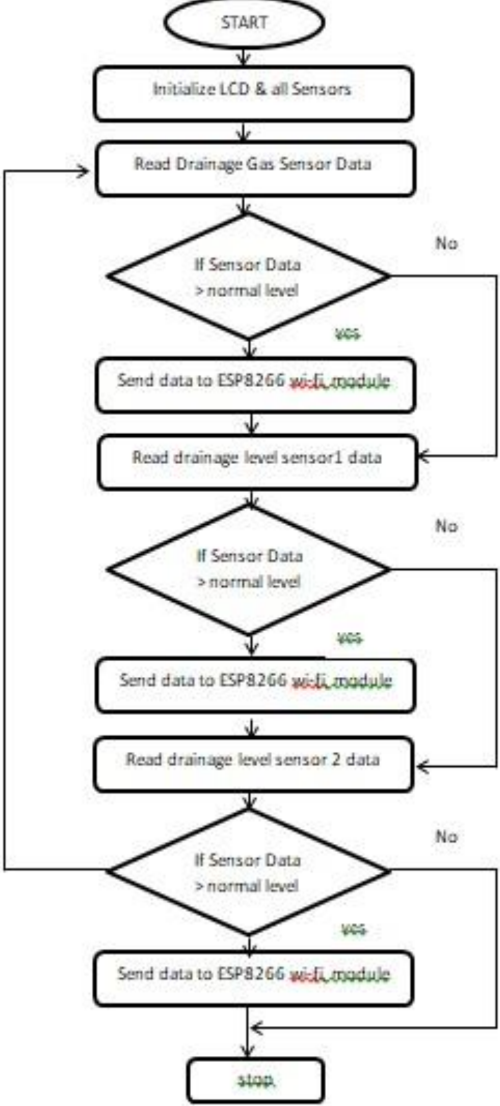


Figure Of Flowchart

Result

- When the system get started by taking power supply externally all the sensor will start working
- The gas sensor sense the harmful gases and report to microcontroller. The water level detection sensor check the flow of water and send value to microcontroller by using ATmega328P microcontroller and ESP 8266 Wi-Fi module output is shown on blynk application.

9 Conclusion

Our project helps to reduce the problem of drainage system with the help of sensors like water level detection sensor and gas sensor our mechanism helps to notify the connected network, when the harmful gases are detected to gas sensor and water level is detected by water level detection sensor with help of ATmega328P microcontroller and ESP8266 Wi- Fi module which is connected with the Blynk server. by this project the underground drainage system can be easily organized.

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Automatic Barricade with Traffic Light

[1]Payal Londhe ,[2]Prasad Gujar ,[3]Abhishek Sungar,[4]Komal Andhare,[5]Akanksha Ramugade

[1,2,3,4,5] UG Students, Department of Electronics and Telecommunication Engineering Yashoda Technical Campus Wadhe,Satara ,India

ABSTRACT

India is the second largest road network in the world.Traffic jams occur due to drivers violating traffic rules. One of the major reasons of accidents is violation traffic signals. In this project we are going to implement the Automatic barricade with traffic light using Arduino UNO R3 . Till date traffic controlling system was based on traffic police and electronic signals. Hence it necessary to implement the Automatic barricade with traffic light system for the purpose of reducing accidents and smooth moving of traffic.

Implementation of the Automatic barricade with traffic light system will ensure that driver will have to compulsorily follow traffic signals and violation of traffic signals will not be possible

Keywords: Traffic control, Arduino , Traffic Signal, Barricade..

1 INTRODUCTION

Safety begins right from our self, so it is at most important to follow traffic rules and regulations.

The focus of this project is to improve the current traffic system and reduce this grave issue of traffic congestion to some extent.

We have considered here a four lane road. To stop the people from breaking the signals we will install 4 barricades which will block the road once the signal turns red.

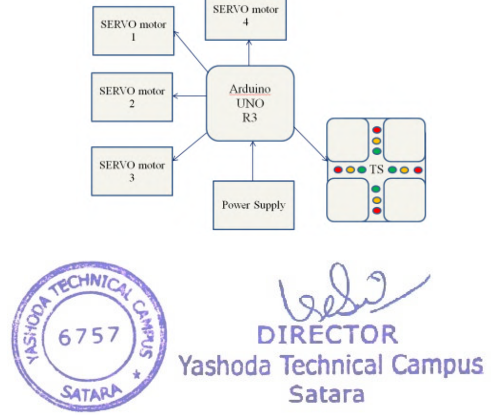
Red LED indicates CLOSED , Yellow LED indicates driver be ready to drive or be ready to stop and green LED indicates OPEN..

2 PROBLEM DEFINITION

The problems which are encountered in the previous identification systems are:

- We know that the yellow light is getting on at least two time , one is for get ready to drive and second is get ready to stop.
- For that we will keep the message plate as " Go Slow ".

3 BLOCK DIAGRAM



4 HARDWAREDESCRIPTION-

ARDUINO UNO-

The Arduino Uno is a microcontroller board based on the Arduino328 (datasheet). It has 14 digital input/output pins (of which 6 can be used as PWM outputs), 6 analog inputs, a 16 MHz crystal oscillator, a USB connection, a power jack, an ICSP header, and a reset button. It contains everything needed to support the microcontroller; simply connect it to a computer with a USB cable or power it with a AC-to-DC adapter or battery to get started. The Uno differs from all preceding boards in that it does not use the FTDI USB-to-serial driver chip. Instead, it features the Arduino8U2 programmed as a USB-to-serial converter. "Uno" means one in Italian and is named to mark the upcoming release of Arduino 1.0. The Uno and version 1.0 will be the reference versions of Arduino, moving forward

SERVO MOTOR SG90-

The Tower Pro SG90 is a simple Servo Motor which can rotate 90 degrees in each direction (approximately 180 degrees in total) , and it operates on 5V.

BARRICADE-

Barricade is any object or structure that creates a barrier or obstacle to control, block passage or force the flow of traffic in the desired direction

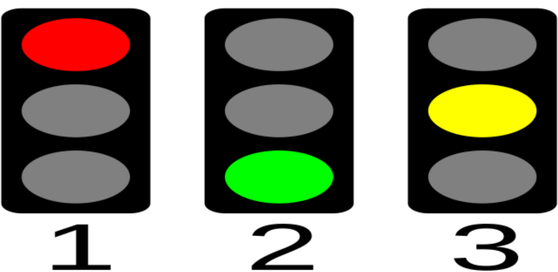
TRAFFIC SIGNAL –

In simple words, traffic lights usually change in this order:

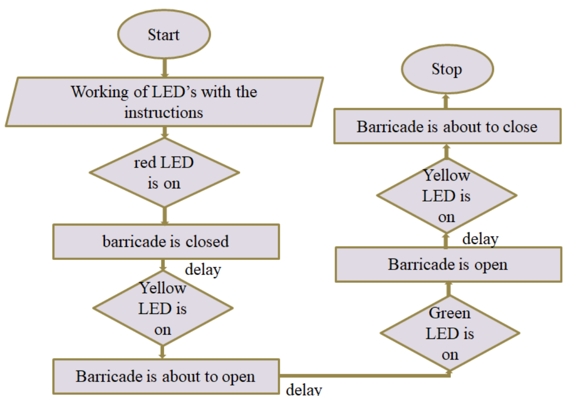
Red light on: This tells drivers to stop.

Green light on: This means the driver can start driving or keep driving.

Yellow light on: This tells drivers to stop when it is safe to, because the light is about to turn red OR ready to drive because the light is about to green .

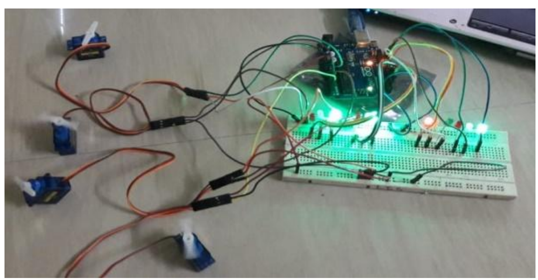


5 WORKING PRINCIPLE WITH FLOWCHART –



5 RESULT-

The result of our project is , when the red light is on barricade is in down position .
when yellow led will glow after red signal it will be sign of be ready to go .
once the led get's green it will be in open position and here yellow led glow once again that will be sign of be ready to stop.
This process is continuously happen with all lanes and automatically the frequency of accidents due to traffic breaking will be reduce.



6 ADVANTAGES & DISADVANTAGES

- Help's in reducing the frequency of an accident's.
- This system will also boost the mission of "Smart City".
- Provide authority to the drivers to move with confidence.
- Will reduce traffic police work load.

- Direct traffic on different routes without excessive congestion.

DISADVANTAGES-

- This is only useful for 2 way road's.

7 COCLUSION-

- Very effective in controlling traffic.
- This will help in betterment of smooth moving traffic.
- It ensure that drivers will not face traffic jams and enjoy the drive.
- The issues of accident can be reduced drastically.

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REVIEW ARTICLE

Review on Microwave, The General purpose in Microwave Assisted Synthesis for Green Chemistry

Aishwarya A Jadhav, Rasika P. Devale
Department of B Pharmacy, Yashoda Technical Campus, Satara.
*Corresponding Author E-mail: **jadhavaishwaryaa@gmail.com**

ABSTRACT:
In this review we explain all the detailed information about Microwave assisted synthesis. Now a days the Microwave very much beneficial in to Microwave assisted synthesis reaction for green chemistry work by the various reactions. this is initially used by the save energy and rate of reaction is fast. Microwave synthesis capable of predicting many properties and rate of synthesis reaction is fast in small period of time to get from product. all type chemical reaction synthesis is also done by this microwave. various authors words on their subject by using this Microwave assisted synthesis. I show interest into microwave because of this is very beneficial for performing synthesis of reaction. In microwave various principals are added and this will be beneficial or helpful to guide scientist.

KEYWORDS: Microwave assisted synthesis, green chemistry, Microwave.

1. INTRODUCTION:

Green chemistry is defined as environmentally benign chemical synthesis.in that microwave is a general-purpose green chemistry for performing reaction in small period of time. Microwave initially started used in or released in 1986 by the groups of Gedye and Giguere/ Majetich although the use is microwave heating in chemical purpose can be back to 1950. And originates from scientist Gedye and Giguere started his research microwave synthesis in the using green chemistry. if focusses on a process whether carried out in industry or chemical laboratory. the reduced the use and generation of harmful substance or byproduct. Green chemical deals with environmentally for chemical synthesis to devise pathway for the prevention of pollution according to Paul T. Anastas[1]

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Table no: 1

Original author	**Gedye and Giguere**
Developers	America
Initial release	1946
Stable release back to chemical purpose	1950
Website	www.microwaveassistedsynthesis.com

We will use the microwave assisted synthesis in windows environment. microwave is capable for predicting many properties of metal catalysis and heating principle. all types chemical synthesis reaction including the following

- Basic principle of microwave assisted synthesis.
- Theoretical aspects of microwave dielectric heating.
- Microwave accelerated metal catalysis.
- Heterocyclic chemistry using microwave assisted approaches.
- Microwave assisted reduction.
- Microwave assisted multi component reactions.
- Integrating microwave assisted synthesis and solid supported reagents.
- Microwave assisted solid phase synthesis.
- Scale up of microwave assisted organic synthesis[1,2]

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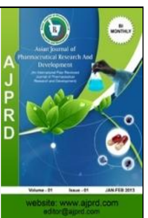
Available online on 15.08.2021 at http://ajprd.com



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Research Article

Evaluation of Anti-hyperlipidemic Activity of Red Onion In Experimental Animals

Pooja Kadam *[1], Vitthal Chaware [2], Vivek Redasani [3]

[1]Department of Pharmacology, YSPM's Yashoda Technical Campus Wadhe, Satara, India.

[2]Head of Department (Pharmacology), YSPM's Yashoda Technical Campus, Wadhe, Satara, India.

[3]Principal, YSPM's Yashoda Technical Campus, Wadhe, Satara, India.

ABSTRACT

Objective: To evaluate antihyperlipidemic effect of red onion on poloxamer 407 induced hyperlipidemia in wistar albino rats.

Methods: Hyperlipidemia was induced by intraperitoneal injection of poloxamer-407 (P-407) at a dose of 1.0g/kg body weight in wistar albino rats. Drug treatments were done by oral gavage for 21 days. At the end of the study, animals were kept fasted over night and then blood samples were collected. The serum total cholesterol (TC), triglycerides (TG), and High density lipoprotein (HDL) were measured while low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were calculated by Friedewald formula and atherogenic index was also calculated.

Results: From the present investigation, it was observed that ethanolic extract of red onion have shown significant reduction in serum cholesterol, triglyceride and lipoprotein levels and increase in HDL level in P-407 induced hyperlipidemia.

Conclusion: The findings in this study revealed the effectiveness of ethanolic extract of red onion against hyperlipidemic activity.

Key words: Hyperlipidemia, Poloxamer 407, red onion, Atorvastatin, Lipid Profile

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***Address for Correspondence:**

P. B. Kadam, Department of Pharmacology, YSPM's Yashoda Technical Campus Wadhe, Satara, India.

INTRODUCTION:

Hyperlipidemia is also termed as acquired hyperlipoproteinemia; high blood triglycerides; high blood cholesterol; high cholesterol; high triglycerides; hyperlipidemia etc. [1]. It is an elevation of one or more of the plasma lipids, including triglycerides, cholesterol, cholesterol esters and phospholipids etc. [2]. It is also described by elevation of serum total cholesterol and low density and very low-density lipoprotein cholesterol and decreased high-density lipoprotein levels [3–6]. Number of clinical trials have verified that increase in plasma total cholesterol (TC) and triglycerides (TG) levels are implicated in the development of atherosclerosis [2]. It is also one of the important risk factor for developing cardiovascular diseases (CVDs) [4,7,8]. Principal CVDs associated with hyperlipidemia are hypertension, ischemic heart diseases, stroke, coronary heart diseases (CHDs) and atherosclerosis. They account for almost 80% of the burden of CVD in both developed and developing countries. A 20% decrease in blood cholesterol level can reduce about 31% of CHD incidence, and 33% of its mortality rate [9]. Because of all these risks associated with hyperlipidemia, treatment is often recommended for people with hyperlipidemia [10].

Effective treatment of hyperlipidemia includes dietary modifications and medications. There are number of antihyperlipidemic agents available in the market but they show certain side-effects and contraindications [10]. Statins are the first-line drugs for treatment of hyperlipidemia which act by inhibiting 3-hydroxy-3-methyl-glutaryl-

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coenzyme A (HMGCoA) reductase. However, statins have some adverse effects including rhabdomyolysis and derangements in hepatic function. Fibrates are used as second-line drugs for the treatment of dyslipidemia which acts by activating peroxisome proliferator-activated receptor alpha. However, fibrates shows adverse effects like allergic reactions, nausea, diarrhea etc. Nicotinic acid is also used to treat hyperlipidemia. However, it causes flushing, nausea, vomiting, diarrhea, anorexia like side-effects. Ezetimibe and bile acid sequestrants shows hypolipidemic effect by decreasing intestinal cholesterol absorption; but these drugs are associated with increased gastrointestinal adverse events and also affect the absorption of other biologically important substances [11]. An herbal treatment for hyperlipidemia has no side effects and is relatively less costly, locally available [4]. Hence, people are more interested towards traditional medicinal plants due to their natural origin, safe and non-toxic nature [12].

Red onion (*Allium cepa* L.) is the most widely cultivated species of the genus Allium. The portion of the plant commonly used is the bulb, utilized as a food ingredient to give flavour and aroma to a large variety of dishes [13]. Red onion has been reported to contain flavonoids, phenols, tannins, triterpenoids, cardiac glycosides, saponin and steroid phytochemicals [14]. It also contains Quercetin, cycloalliin, S-methyl-L-cysteine, S-propyl-L-cysteine sulfoxide, dimethyl trisulfide, S-methyl-L-cysteine sulfoxide etc. Literature survey indicates that red onion possesses anti-diabetic [15], Anti-Obesity [16], Hepatoprotective [17], Antidepressant [18], Analgesic [19], Anti-inflammatory [19] and antimicrobial activity etc [14]. Therefore, the present investigation was undertaken to evaluate hypolipidemic effect of ethanolic extract of red onion in poloxamer 407 induced hyperlipidemic rats which has not been previously reported. It is our belief that this investigation will take us another step forward in our quest to understand the mechanism of action of red onion in prevention and treatment of arteriosclerosis and heart related diseases.

MATERIALS AND METHODS:

Drugs & chemical

Poloxamer 407 was acquired from Emcure Pharmaceuticals Ltd Pune. Atorvastatin (Lipvas 20, cipla ltd.) was purchased in a tablet form at strength 20 mg. All other chemicals and reagents used were of analytical grade and procured from approved chemical suppliers. The total cholesterol (TC), triglycerides (TG) and high-density lipoprotein (HDL) were measured with the help of commercial kits.

Collection and extraction of plant material

Fresh red onions were purchased from a local market, satara and were identified by a botanist. They were washed with tap water and then cut into medium pieces. The chopped onions were then blended. The blended onion (200 g) then macerated in 2000 ml ethanol and allowed to stand for a period of 72 h. It was filtered using Whatman filter paper (No. 1). The filtrate was concentrated at 40°C in a water bath for complete dryness. Crude extract obtained was stored at 4°C for further use [20].

Experimental animals

The complete experiment was carried out using 36 wistar albino rats of either sex weighing 150 -200g. The study protocol was approved by Institutional Animal Ethics Committee (IAEC) of YSPM, Satara. The animals were procured from registered breeder and acquainted in the quarantine area for one week. Animals were housed in clean polypropylene cages in a controlled room temperature 22°C ± 2°C, relative humidity of 50 ± 15% and 12 hr dark/ 12 hr light cycle at our Institution's animal house and allowed to acclimatize for two weeks. The animals were fed with standard pellet diet and water *ad libitum*. Animals were maintained as per Committee for the Purpose of Control and Supervision of Experiments on Animals Guidelines.

Preparation of standard drug

Atorvastatin tablets were crushed into powder, dissolved in distilled water at dose 10mg/kg b. w. and administered orally *ad libitum* [9,21].

Induction of hyperlipidaemia

Poloxamer 407 dissolved in cold distilled water at dose 1.0g/kg b. w. and introduced intraperitoneally. All syringes were placed on ice prior to P-407 administration to maintain the polymer in a mobile viscous state during the injection [9,21].

Experimental design

A total 36 wistar albino rats of either sex were randomly divided into 6 groups containing 6 animals in each group. Group 1 (Normal control) did not receive any treatment apart from vehicle 10ml/kg b. w. /day for 21 days. Group 2 (Hyperlipidemic control) were induced with 1.0g/kg b. w. dose of P-407 without treatment [22]. Group 3 (Standard control) were induced with 1.0g/kg dose of P-407 and treated with atorvastatin at a dose of 10mg/kg b. w. /day for 21 days. Group 4, 5 and 6 were induced with 1.0g/kg dose of P-407 and treated with test drug at dose 200 (low), 300 (medium) and 400 (high) mg/kg b. w. /day for 21 days respectively [21].

Blood Sample Collection

At end of the experimental period, animals were kept fasted over night and anaesthetized with diethyl ether. Blood samples were collected serially by retro orbital puncture. The blood was allowed to clot for 30 min at room temperature then serum was separated by centrifugation and used for lipid analysis.

Evaluation parameters

Body weight

Body weight were recorded on the first day of treatment of all groups and final body weight were taken at the end of treatment of all groups to calculate changes between the initial and final body weight of animal throughout the study.

Biochemical parameters

The resulting serum was analyzed for serum TC and TG by Quinoneimine dye absorption method at 505 nm [23] and HDL by precipitation with phosphotungstic acid and Magnesium chloride [24].

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Very low density lipoprotein cholesterol (VLDL-C) was calculated as [11,25,12]:

VLDL = TG/5.

Low density lipoprotein cholesterol (LDL-C) levels were calculated using Friedewald's formula [25]:

LDL = TC - HDL - TG/5

The atherogenic index (A.I.) was calculated using the following formula [26]:

(A.I.) = LOG (TG/HDL)

Liver histopathology

The fixed specimens of liver were processed by washing through running tap water, dehydration through ascending grades of alcohol, clearing through xylene and embedding completely with in paraffin into blocks. The serial sections of not exceeding 3 mm thickness were cut using microtome and were mounted on polylysine coated slides, deparaffinised using xylene, rehydrated and stained with hematoxylin and eosin, dehydrated, cleared and mounted on DPX under glass cover slips. The slides were then observed under light microscope which was connected to a camera to capture images.

Statistical analysis

The results were expressed as Mean ± SEM (n=6). The statistical analysis was carried out with Graph pad prism 5.0 software. The data was statistically analyzed using one-way ANOVA followed by Tukey's multiple comparison tests and $p < 0.05$ was considered to be statistically significant.

RESULTS & DISCUSSION

RESULTS

Yield of the extract

The yield of the extract was found to be 3.7%. Further preliminary phytochemical screening revealed the presence of flavonoids, saponins, phenol, diterpenes, triterpenes, alkaloids, phytosterol and proteins.

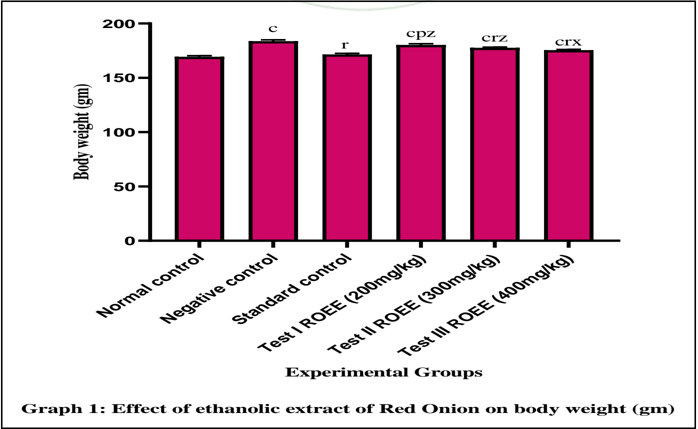
Body weight

Effect of administration of ethanolic extract of red onion on body weight of experimental animals has been shown in table 1. When compared with normal control group, negative control group and all test group animals showed significant ($p<0.001$) increase in body weight but standard control group animals do not show any significant changes in body weight. Furthermore, Standard control group and test II and III group animals showed significant ($p<0.001$) reduction in body weight, whereas test I group showed significant ($p<0.05$) reduction in body weight as compared to negative control group.

Table 1: Effect of ethanolic extract of red onion on body weight of P-407 induced hyperlipidemia in experimental animals.

Sr.	Experimental group	Initial body weight (gm)	Final body weight (gm)	Change in body weight (gm)
1	Normal control	166.6 ± 1.186	169.4 ± 1.622	4.642 ± 1.284
2	Negative control	170.30 ± 3.963	183.8 ± 1.126 ^{c}	13.45 ± 1.098
3	Standard control	168.9 ± 2.032	171.6 ± 1.287 ^{r}	5.33 ± 1.305
4	Test I (200mg/kg)	170.74 ± 2.889	180.3 ± 1.425^{cpz}	9.615 ± 1.491
5	Test II (300mg/kg)	168.75 ± 2.067	177.4 ± 0.689^{crz}	8.03 ± 1.56
6	Test III (400mg/kg)	169.15 ± 1.833	175.5 ± 0.872^{crx}	7.28 ± 1.046

Normal control: distilled water; Negative control: Poloxamer 407; Standard control: Atorvastatin; Test I: ROEE (200mg/kg); Test II: ROEE (300mg/kg); Test III: ROEE (400mg/kg).



Graph 1: Effect of ethanolic extract of Red Onion on body weight (gm)

Values represented mean ± SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. a<0.05, b<0.01, c<0.001; Data compared with normal control; p<0.05, q<0.01, r<0.001 Data compared with negative control; x<0.05, y<0.01, z<0.001 Data compared with standard control (by one way ANOVA followed by Tukey's multiple comparison test)

Serum lipid profile

Poloxamer 407 administration developed acute hyperlipidemia in rats by significantly increasing the level of total cholesterol, triglycerides, lipoproteins and decreasing HDL level as compared to normal control group.

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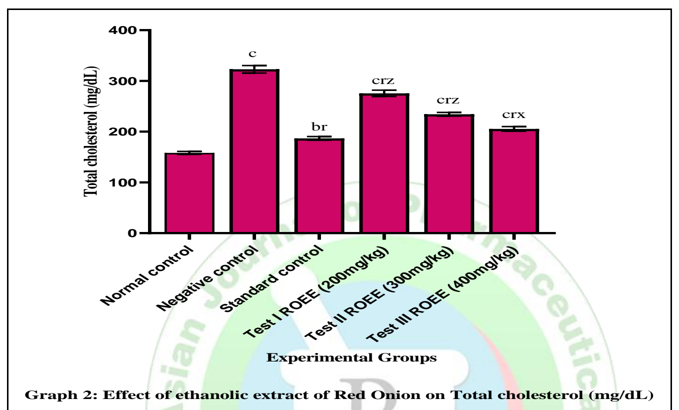
Table 2: Effect of ethanolic extract of red onion on serum lipid profile of P-407 induced hyperlipidemia in experimental animals.

Sr. No.	Experimental group	TC (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	Atherogenic index
1	Normal control	158.3 ± 2.934	105.0 ± 2.061	45.00 ± 1.773	92.25 ± 3.382	21.00 ± 0.4522	0.3691 ± 0.01367
2	Negative control	323.0 ± 7.431 ^{c}	207.4 ± 5.259 ^{c}	23.08 ± 0.740 ^{c}	258.4 ± 6.506 ^{c}	41.48 ± 1.052 ^{c}	0.9541 ± 0.01793 ^{c}
3	Standard control	186.7 ± 3.669 ^{br}	128.80 ± 3.560 ^{ar}	38.02 ± 1.003 ^{br}	123.0 ± 3.420 ^{cr}	25.76 ± 0.7119 ^{ar}	0.5298 ± 0.02173 ^{cr}
4	Test I (200mg/kg)	275.6 ± 6.107 ^{crz}	185.7 ± 6.401 ^{cpz}	28.96 ± 0.879 ^{cpz}	209.5 ± 5.120 ^{crz}	37.15 ± 1.280 ^{cpz}	0.8067 ± 0.01864 ^{crz}
5	Test II (300mg/kg)	234.3 ± 3.614 ^{crz}	166.3 ± 5.921 ^{crz}	30.57 ± 1.386 ^{cqy}	170.40 ± 2.324 ^{crz}	33.26 ± 1.184 ^{crz}	0.7364 ± 0.01412 ^{crz}
6	Test III (400mg/kg)	207.8 ± 4.021 ^{crx}	150.9 ± 3.912 ^{crx}	31.55 ± 1.218 ^{cry}	146.1 ± 4.276 ^{crx}	30.18 ± 0.782 ^{crx}	0.6805 ± 0.01656 ^{crz}

Normal control: distilled water; Negative control: Poloxamer 407; Standard control: Atorvastatin; Test I: ROEE (200mg/kg); Test II: ROEE (300mg/kg); Test III: ROEE (400mg/kg).

Effect of ethanolic extract of red onion on Total cholesterol

Table 2 shows that the ethanolic extract of red onion has significantly affected the level of total cholesterol in hyperlipidemic rats. The results found that the negative control group showed significant (p<0.001) increase in the level of TC as compared to normal control group. Whereas standard control, test I, test II and test III groups showed significant (p<0.001) reduction in TC level when compared with negative control group.

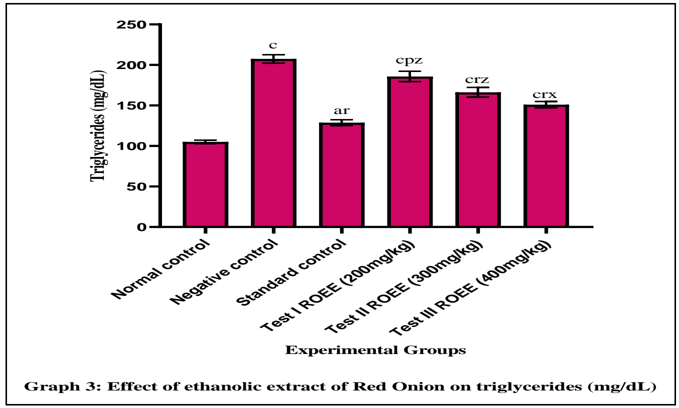


Graph 2: Effect of ethanolic extract of Red Onion on Total cholesterol (mg/dL)

Values represented mean ± SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. a<0.05, b<0.01, c<0.001; Data compared with normal control; p<0.05, q<0.01, r<0.001 Data compared with negative control; x<0.05, y<0.01, z<0.001 Data compared with standard control (by one way ANOVA followed by Tukey's multiple comparison test).

Effect of ethanolic extract of red onion on Triglycerides

The mean values for triglyceride level are given in table 2. Negative control group showed significantly (p<0.001) increased level of triglycerides as compared to normal control group. Standard control, test II and test III group showed significant (p<0.001) reduction and test I group showed significant (p<0.05) reduction in the level of triglycerides as compared to negative control group.



Graph 3: Effect of ethanolic extract of Red Onion on triglycerides (mg/dL)

Values represented mean ± SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. a<0.05, b<0.01, c<0.001; Data compared with normal control; p<0.05, q<0.01, r<0.001 Data compared with negative control; x<0.05, y<0.01, z<0.001 Data compared with standard control (by one way ANOVA followed by Tukey's multiple comparison test).

Effect of ethanolic extract of red onion on HDL

According to the data presented in table 2, the negative control group showed significant (p<0.001) decrease in the level of HDL as compared to normal control group. Whereas significant increase in the level of HDL was observed in standard control (p<0.001), test I (p<0.05), test

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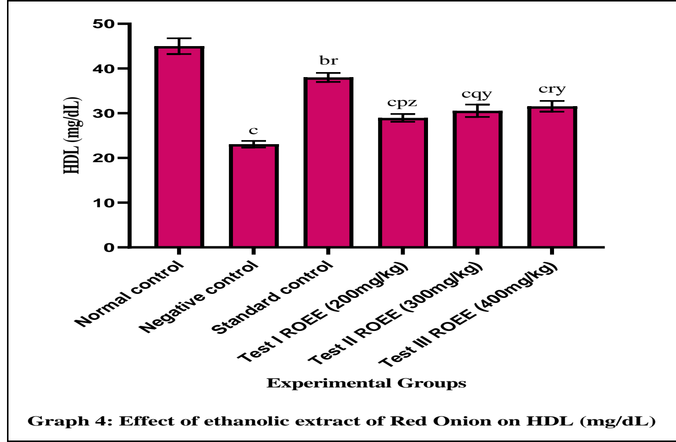
II ($p<0.01$) and test III ($p<0.001$) group when compared with negative control group.

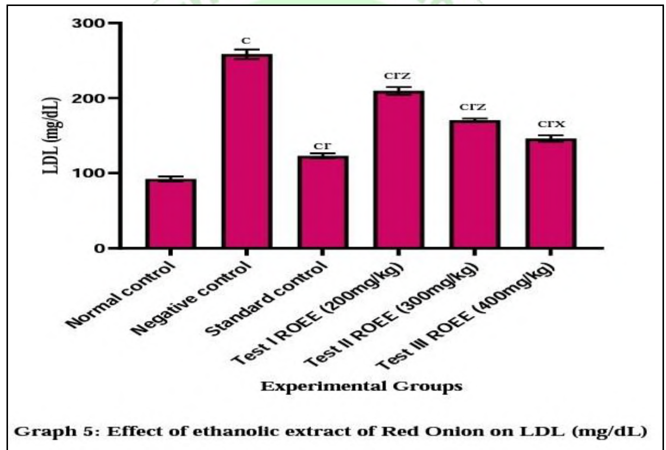


Graph 4: Effect of ethanolic extract of Red Onion on HDL (mg/dL)

Values represented mean ± SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. a<0.05, b<0.01, c<0.001; Data compared with normal control; p<0.05, q<0.01, r<0.001 Data compared with negative control; x<0.05, y<0.01, z<0.001 Data compared with standard control (by one way ANOVA followed by Tukey's multiple comparison test).

Effect of ethanolic extract of red onion on LDL

The mean values for LDL levels in normal and hyperlipidemic groups are given in table 2. When compared with normal control group negative control group showed significant ($p<0.001$) increase in the LDL level. While as compared to negative control group the standard control, test I, II and III group showed significant ($p<0.001$) decrease in LDL level.



Graph 5: Effect of ethanolic extract of Red Onion on LDL (mg/dL)

Values represented mean ± SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. a<0.05, b<0.01, c<0.001; Data compared with normal control; p<0.05, q<0.01, r<0.001 Data compared with negative control; x<0.05, y<0.01, z<0.001 Data compared with standard control (by one way ANOVA followed by Tukey's multiple comparison test).

Effect of ethanolic extract of red onion on VLDL

Table 2 illustrated that the level of VLDL in different treatment groups was considerably affected. The negative control group showed significant ($p<0.001$) increase in the level of VLDL as compared to normal control group. Whereas test I group showed ($p<0.05$) and standard control, test II and III showed ($p<0.001$) significant reduction in VLDL level as compared to negative control group.

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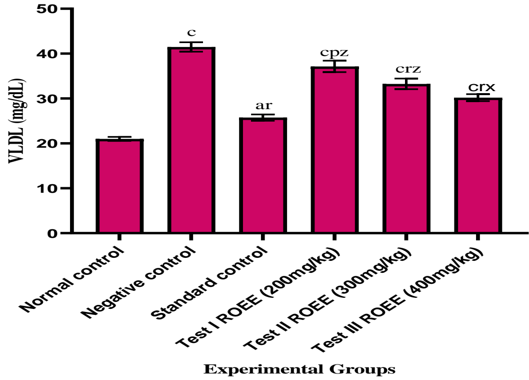
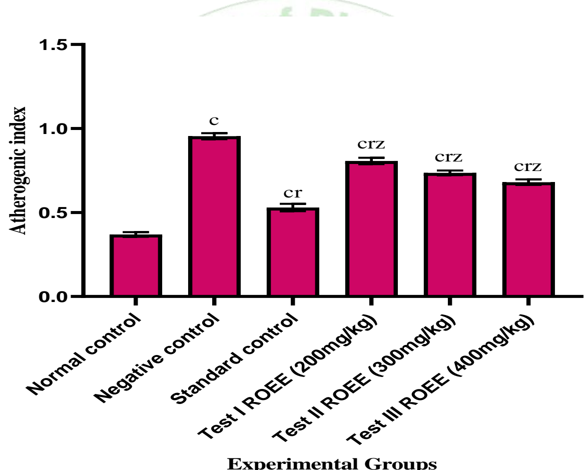


Graph 6: Effect of ethanolic extract of Red Onion on VLDL (mg/dL)

Values represented mean ± SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. a<0.05, b<0.01, c<0.001; Data compared with normal control; p<0.05, q<0.01, r<0.001 Data compared with negative control; x<0.05, y<0.01, z<0.001 Data compared with standard control (by one way ANOVA followed by Tukey's multiple comparison test).

Effect of ethanolic extract of red onion on Atherogenic index

According to the data presented in table 2 the negative control group showed significant ($p<0.001$) increase in the atherogenic index as compared to normal control group. Whereas standard control and all test groups showed significant ($p<0.001$) reduction in atherogenic index as compared to negative control group.



Graph 7: Effect of ethanolic extract of Red Onion on atherogenic index

Values represented mean ± SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. a<0.05, b<0.01, c<0.001; Data compared with normal control; p<0.05, q<0.01, r<0.001 Data compared with negative control; x<0.05, y<0.01, z<0.001 Data compared with standard control (by one way ANOVA followed by Tukey's multiple comparison test).

Histopathological changes

Histopathology of the liver for normal control, negative control and test III group were carried out

Histopathological observation of liver in normal control group

The normal control group animals showed normal hepatocyte architecture such as healthy nucleus and parenchymal structure [Fig.1 (a) & (b)].

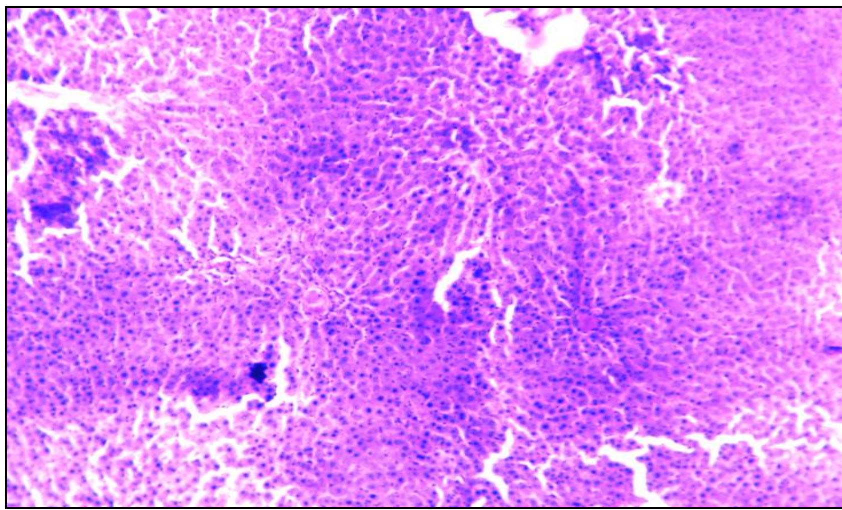


Figure 1 (a): Liver section (100X) of normal control group showing normal hepatocytes

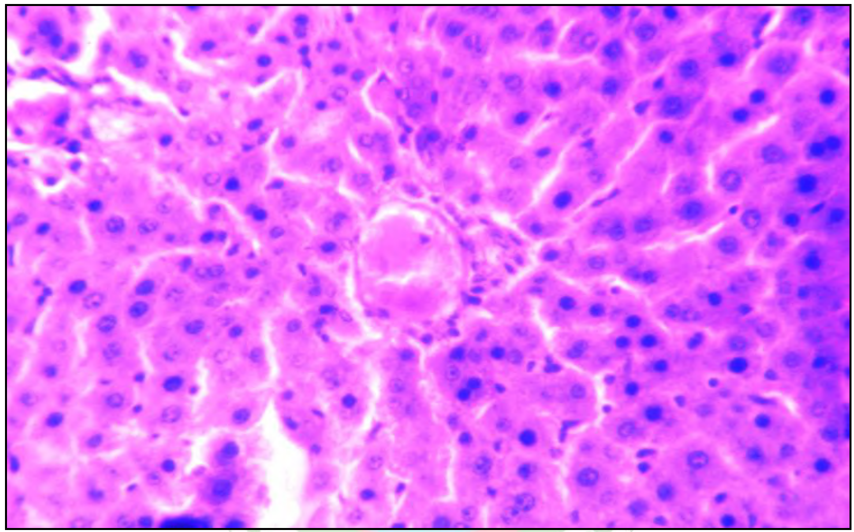


Figure 1 (b): Liver section (400X) of normal control group showing normal hepatocytes

Histopathological observation of liver in negative control group

As compared to normal control group the negative control group animals showed fatty changes, altered hepatocyte architecture along with necrosis, congestion and leucocytic infiltration [Fig.2 (a) & (b)].

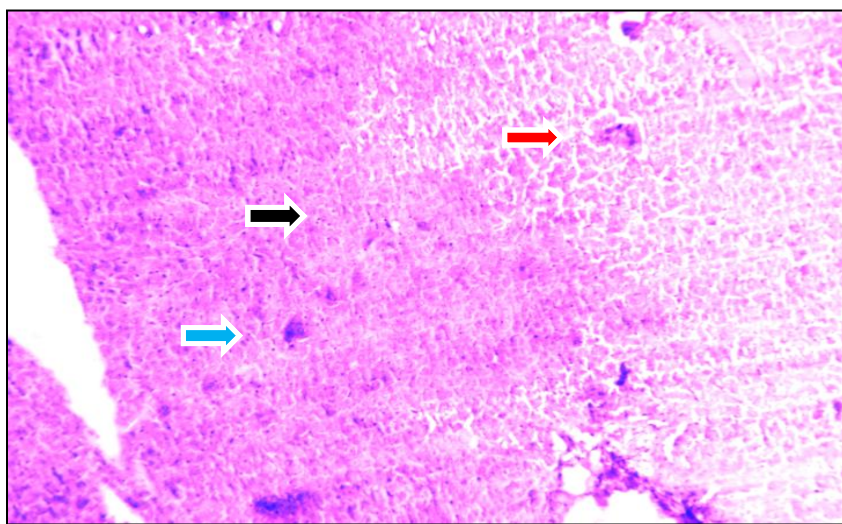


Fig 2 (a). Liver section (100X) of negative control group showing necrosis (Black arrow), congestion (red arrow) and leucocytic infiltration (blue arrow)

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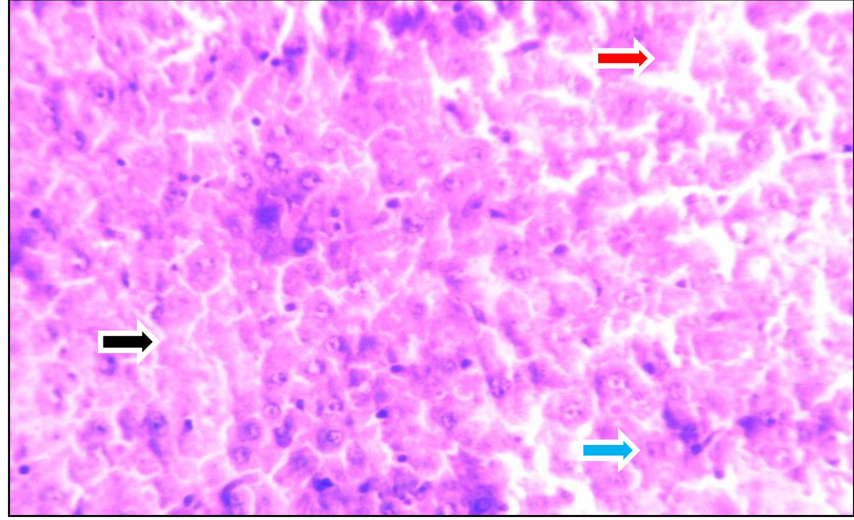

Figure 2 (b). Liver section (400X) of negative control group showing necrosis (Black arrow), congestion (red arrow) and leucocytic infiltration (blue arrow)

Histopathological observation of liver in test III group

When compared with negative control group the test III group animals has reduced fatty changes and restored the hepatocytes near to the normal group [Fig.3 (a) & (b)].

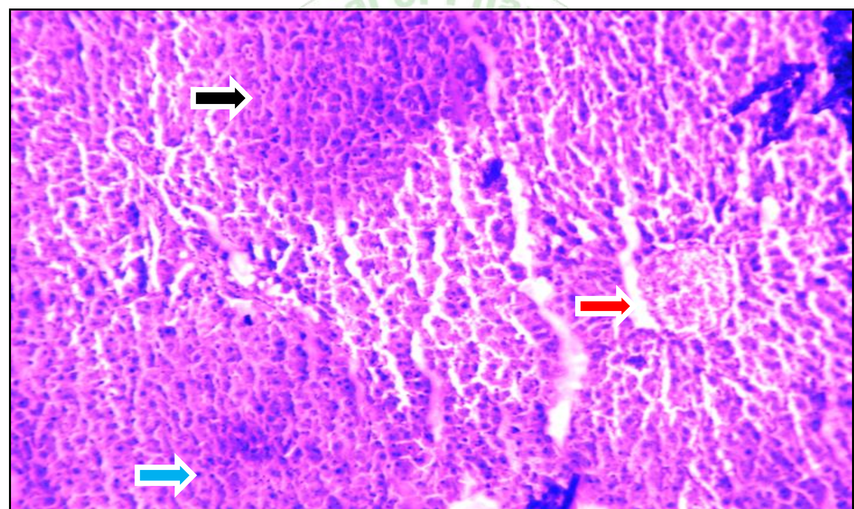

Figure 3 (a): Liver section (100X) of test III group showing necrosis (Black arrow), congestion (red arrow) and leucocytic infiltration (blue arrow)

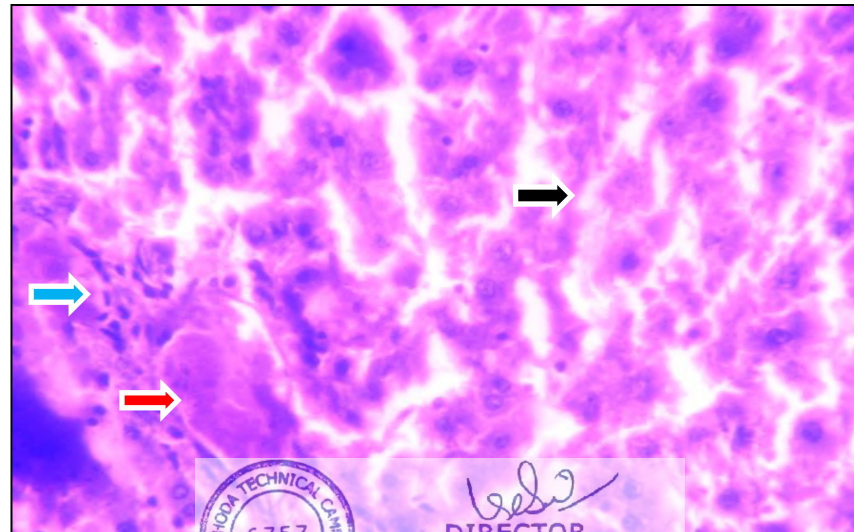

Figure 3 (b): Liver section (400X) of test III group showing necrosis (Black arrow), congestion (red arrow) and leucocytic infiltration (blue arrow)

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DISCUSSION

Lipids are organic compounds which are water insoluble but soluble in organic solvents. Lipids perform number of functions such as chemical messengers, storage and provision of energy, maintenance of temperature and membrane lipid layer formation. Hyperlipidemia is nothing but abnormally elevated level of lipids such as total cholesterol (TC), triglyceride (TG) and lipoproteins [26]. Diseases associated with hyperlipidemia are major risk factors for development of cardiovascular diseases (CVD) [27]. Hyperlipidemia is risk factor for onset and progression of atherosclerosis [28,29] viz high risk factor in development of coronary heart diseases [26]. Hence prevention or treatment of such disorders can be achieved by targeting the causative hyperlipidemia [27].

P – 407 induced hyperlipidemia is one of the animal model used for the evaluation of antihyperlipidemic activity of drug. It was harmless to membranes of cells, in earlier studies it was used effectively to induce hyperlipidemia. Poloxamer 407, a non-ionic synthetic copolymer surfactant commonly known used to induce hyperlipidemia in small laboratory animals within 24 h through i. p. injection. Due to its rapid onset, convenience, reproducibility, and lack of undesirable toxicity, P-407 was used in this study to induce hyperlipidemia in animals [11,30]. A single injection of P-407 caused elevations of serum cholesterol and triglyceride levels in rats. P-407 induced hyperlipidemia via alterations in activity of 3-hydroxy-3- methylglutaryl-CoA (HMG-CoA) reductase, lipoprotein lipase (LPL), lecithin cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP), hepatic lipase (HL) and lipoprotein lipase (LPL). P-407 directly inhibits the capillary (heparin releasable) LPL and HL, and it indirectly increases the biologic activity of CETP and LCAT [9,31].

In our analysis, there was marked increase in the level of total cholesterol, triglycerides, LDL,VLDL, AI and decrease in the level of HDL in negative control group as compared to normal control group (Table 2) confirming that i.p. injection of P-407 has induced hyperlipidemia experimentally [9]. Red Onion ethanolic extract (200mg/kg, 300mg/kg and 400mg/kg) significantly decreased the increased level of total cholesterol, triglycerides, LDL,VLDL, AI and increased the level of HDL after treatment suggest the ameliorative potential of Red Onion.

In our analysis the body weight gain of different groups of rats showed that negative control group animals showed the significant increase in the body weight as compared to normal control group animals. After treatment with standard and test drug the body weight decreased significantly.

In this study, the significant elevation of TC concentration was achieved by the indirect stimulation of HMG CoA reductase by an intraperitoneal (i.p) injection of P-407 [26]. Hence the hypocholesterolemic effect of red onion ethanolic extract could be due to decreased activity of hepatic HMG CoA reductase, stimulation of Cholesterol-7-alpha-hydroxylase, which converts cholesterol into bile acids. It could also be due to the presence of saponins, a phytochemical which forms insoluble complexes with cholesterol or their bile salt precursor, thus making them unavailable for absorption. The results obtained in our analysis conform to earlier report that polyphenols possesses antilipidemic activity [9,26]. Besides, the standard drug (Atorvastatin) used in this study inhibits HMG CoA reductase, viz a rate limiting enzyme in the biosynthesis of cholesterol [26].

In our analysis, elevation in TG concentration after P-407 i.p. injection results primarily from an inhibition of TG degradation, P-407 directly inhibits capillary lipoprotein lipase (LPL) enzyme which is responsible for plasma TG hydrolysis and its clearance from the circulation [11,26]. The Red Onion ethanolic extract could have reduced TG levels by either activating lipoprotein lipase enzyme which hydrolyses the triglyceride into fatty acid hence decreasing triglyceride levels or by inhibiting lipolysis so that fatty acids do not get converted to triglyceride [11, 12, 26].

HDL act as cholesterol scavengers, they transport excess cholesterol and cholesterol esters from the blood and peripheral tissues back to the liver where it is broken down to bile acids. It plays a crucial role in reducing blood and peripheral cholesterol concentrations and inhibits formation of atherosclerotic plaque in the aorta therefore known as the protective cholesterol or Good cholesterol [26]. The present study indicates significant elevation in HDL concentration by the standard drug and ROEE. This could possibly be due to increasing activity of lecithin-cholesterol acyl transferase (LCAT), an enzyme which is responsible for incorporating free cholesterol into HDL there by promoting reverse cholesterol transport and competitively inhibiting the uptake of LDL-c by endothelial cells.

LDL (low density lipoprotein) transports cholesterol to the body cells. It transports near 60-70% of total cholesterol to the body cells. Therefore, an increase in TC level accordingly increases LDL-c [26]. LDL is referred as the most dangerous among the serum lipids, and the oxidation of LDL-c leads to its increased penetration of arterial walls. The increased LDL-c levels play a vital role in the development of atherosclerotic lesions that progress from fatty streaks to ulcerated plaques. Therefore, serum LDL levels are used as the basis for initiating and monitoring the treatment of patients with elevated blood cholesterol levels [9]. In the present study, ROEE shows marked reduction in LDL levels (Table 2). This result could be due to the presence of phenolics, a phytochemical which may work by increasing LDL receptors densities in the liver binding to apolipoprotein B thereby making liver cells more efficient to remove LDL from circulation [26].

Very low density lipoproteins (VLDLs) are secreted from the liver. They contain large amount of triglycerides. As it eventually gets converted into LDL and causes buildup of cholesterol on the walls of arteries it is categorized as a type of bad cholesterol [32]. The present study indicates significant decrease in VLDL concentration by the ROEE. This effect could probably be due to the inhibition of triglyceride and possibly fatty acid synthesis by phenolic constituents of red onion. Atherogenic risk predictor index log (TG/HDL-c) has been considered as the most accurate in determining the extent of atherosclerosis and the risk of myocardial infarction. The present study showed that the Red Onion ethanolic extract has significantly reduced

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atherogenic index as compared to negative control group. The results suggest the anti-atherogenic potential of Red Onion ethanolic extract and hence, reducing the development of coronary atherosclerosis [26].

Histopathology study of liver was also carried out to check fatty changes, necrosis of hepatocytes, congestion and leucocytic infiltration. The histopathological report of negative control group animals showed the development of fatty changes, necrosis of hepatocytes, congestion and leucocytic infiltration while the histopathological report of normal control group animals did not show any fatty changes. Whereas the group of animals treated with ROEE (400mg/kg) restored hepatocytes near to normal control group.

CONCLUSION

In conclusion, the present study has demonstrated that ethanolic extract of Red Onion has antihyperlipidemic effect in Poloxamer 407 induced hyperlipidemia. Red Onion ethanolic extract has showed dose dependent activities on body weight, various serum lipids and atherogenic index. Furthermore the better activities has revealed by the ROEE at dose of 400mg/kg. Utilizing this model, Red Onion ethanolic extract was shown to be effective in significantly lowering total cholesterol, triglycerides, LDL, VLDL and increasing HDL cholesterol levels; also decreasing atherogenic index; thus it can be used in the treatment and/or prevention of cardiovascular diseases.

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NH-4, Wadhe Phata, Satara. Tele Fax- 02162-271238/39/40

Website- www.yes.edu.in, Email-registrar_ytc@yes.edu.in

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Criterion III: - Research, Innovations and Extension

Sr. No.	Title of Paper	Name of the Author/s	Name of Journal	Link to article / paper / abstract of the article
1	Review on Guassion, the General Purpose in Computational Chemistry for Medicinal Chemistry	S A Nangare, S H Rohane	Asian J. Research Chem	https://www.ajrconline.org/AbstractView.aspx?PID=2021-14-1-14
2	Review on Discovery Studio: An important Tool for Molecular Docking	S S Pawar, S H Rohane	Asian J. Research Chem	https://www.ajrconline.org/AbstractView.aspx?PID=2021-14-1-14
3	Role of Autodock vina in PyRx Molecular Docking	R P Pawar, S H Rohane	Asian J. Research Chem	https://www.ajrconline.org/AbstractView.aspx?PID=2021-14-2-7
4	Drug Designing in Discovery Studio	B L Jejurikar, S H Rohane	Asian J. Research Chem	https://www.ajrconline.org/AbstractView.aspx?PID=2021-14-2-8
5	Organization of Swiss Dock: In study of Computational and Molecular Docking Study	N S Patil, S H Rohane	Asian J. Research Chem	https://www.ajrconline.org/AbstractView.aspx?PID=2021-14-2-10
6	A Review: Mechanism and Role of Superdisintegrants in the Development of Mouth Dissolving Tablets	V G Raut, P S Nikam, B P Chaudhari, V K Redasani	International Journal of PharmTech Research	https://www.sphinxsai.com/2021/ph_vol14_no1/2/(177-185)V14N1PT.pdf

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7	Evaluation of Protective Role of A Hesperidin on Letrozole Induced Polycystic Ovarian Syndrome (PCOS) in Female Rats	S S Andhalkar, V J Chaware, V K Redasani	International Journal of PharmTech Research	https://www.sphinxsai.com/2021/ph_vol14_no1/abstracts/A(186-198)V14N1PT.pdf
8	Evaluation of Hepatoprotevtive Activity of Leaves Extract of Pithecellobium Dulce in Experimental Animals	K T Sul, VJ Chaware, VK Redasani V J Chaware,	Asian Journal of Pharmaceutical Research And Development	https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Evaluation+of+Hepatoprotevtive+Activity+of+Leaves+Extract+of+Pithecellobium+Dulce+in+Experimental+Animals&btnG=
9	Role of BDNF in Different Neurodegenerative Disease	R J Bhadrike, M M Mali, B P Chaudhari,V K Redasani	International journal of research in pharmacy and pharmaceutical sciences	https://www.pharmacyjournal.in/assets/archives/2021/vol6issue2/6-1-18-860.pdf
10	Evaluation of Antihyperlipidemic Activity of Red Onion in Experimental Animals	P B Kadam, VJ Chaware, VK Redasani	Asian journal of pharmaceutical research and development	file:///C:/Users/admin/Downloads/jmanager,+Journal+manager,+9,+988+pooja,+796,+52-62.pdf
11	Evaluation of Antidiabetic Potential of Eucalyptus globulus Plant Extract in Dexamethasone-Induced Diabetic Rats	U B Kamble, VJ Chaware, V K Redasani	Asian Journal of Pharmaceutical Research and Development	https://www.ajprd.com/index.php/journal/article/view/1021/872
12	Evaluation of Anticataleptic Activity of Hydroxytyrosol on Haloperidol Induced Catalepsy in Experimental Animals	M Bansode, VJ Chaware, V K Redasani	Asian Journal of Pharmaceutical Research and Development	https://www.ajprd.com/index.php/journal/article/view/990/851
13	Insilico Molecular Docking Analysis in Maestro Software	A K Galande, S H Rohane	Asian J. Research Chem	https://www.indianjournals.com/ijor.aspx?target=ijor:ajrc&volume=14&issue=1&article=017

14	Pharmaceutical and Biotechnological Applications of Microsponges as Novel Nano Technological Drug Delivery System	A S Nalawade, S B. Kalbhare, A B Velhal, MJ Bhandwalkar,, R V Jadhav	Advance Pharmaceutical Journal	https://www.apjonline.in/uploaded/p164.pdf
15	A Review on Medicinal Plants of Natural Origin for Treatment of Polycystic Ovarian Syndrome (PCOS)	S S Andhalkar, VJ Chaware, V K Redasani	Asian Journal of Pharmaceutical Research and Development	https://www.ajprd.com/index.php/journal/article/view/949
16	Efficiency of Autodock: Insilico Study of Pharmaceutical Drug Molecules	U M Satpute, S H Rohane	Asian J. Research Chem	https://www.indianjournals.com/ijor.aspx?target=ijor:ajrc&volume=14&issue=1&article=016
17	Study Compaarative Anti-Microbial Activity of Methanolic Extract of Flowers of Calotropis gigantea and Calotropis procera	P B Kadam, D S Kachare, K T Sul	European Journal of Pharmaceutical and Medical Research	NA
18	Liposomes as a carrier for cancer treatment: review	D.S.kachare, R.K.Pawar,P.K.Ghadge,S.S.Mali	Europian journal of P'ceutical and medical research	https://www.researchgate.net/profile/Dhanshri-Kachare-2/publication/342276828_LIPOSOME_AS_CARRIER_FOR_CANCER_TREATMENT_A_REVIEW/links/5eeb73c3a6fdcc73be851db3/LIPOSOME-AS-CARRIER-FOR-CANCER-TREATMENT-A-REVIEW.pdf
19	Role of citrus pectin in biological activity:a review	D.S.kachare, P.K.Ghadge, S.S.Mali	Journal of pharmacovigilance and quality assurance	researchgate.net/profile/Dhanshri-Kachare-2/publication/342276944_Role_of_Citrus_Pectin_in_Biological_Activity_A_Review/links/5eeb77e9299bf1faac5edbec/Role-of-Citrus-Pectin-in-

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				Biological-Activity-A-Review.pdf
20	Formulation and evaluation of herbal scrub using tamarind peel	P.K.Ghadge, Mahamuni S.S., Kachare D.S	International journal of research	file:///C:/Users/Admin/Downloads/1-ijr-july-1657.pdf
21	A Review on Herbal Medicinal Plant for Treatment of Polycystic Ovarian Syndrome	Karishma Yadav Priyanka Ghadge, Aryan Langeh1, Shankar Kalbhare, Prajkta Phadtare, Rupali Bhoite	Asian Journal of Pharmaceutical Research and Development	https://www.ajprd.com/index.php/journal/article/view/799
22	A Review on Antidepressant Activity	Prajkta Phadtare*1, Priyanka Ghadage2, Abhirup Sagare3, Rupali Bhoite4, Karishma Yadav5	Journal of Pharmacology, Toxicology and Therapeutics	NA
23	Review on Antiulcer Activity of Different Herbal Medicines	Rupali Jadhav* Karishma Yadav1 Prajkta Phadtare2 Amruta Tidke3 , Aryan4	Journal of Community Medicine and Pharmaceutical Regulatory Affairs	NA
24	Insilico Molecular docking analysis in Maestro Software	AK Galande, SH Rohane	Asian Journal of Research in Chemistry	https://www.indianjournals.com/ijor.aspx?target=ijor:ajrc&volume=14&issue=1&article=017
25	Review on Guassion, the General Purpose in Computational Chemistry for Medicinal Chemistry	SA Nangare, SH Rohane	Asian Journal of Research in Chemistry	https://www.indianjournals.com/ijor.aspx?target=ijor:ajrc&volume=14&issue=1&article=015
26	Review on Discovery Studio: An important Tool for Molecular Docking	SS Pawar, SH Rohane	Asian Journal of Research in Chemistry	https://www.indianjournals.com/ijor.aspx?target=ijor:ajrc&volume=14&issue=1&article=014
26	Efficiency of AUTODOCK: Insilico study of Pharmaceutical Drug Molecules	UM Satpute, SH Rohane	Asian Journal of Research in Chemistry	https://www.indianjournals.com/ijor.aspx?target=ijor:ajrc&volume=14&issue=1&article=016

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28	Organization of Swiss Dock: In study of Computational and Molecular Docking Study	NS Patil, SH Rohane	Asian Journal of Research in Chemistry	https://www.indianjournals.com/ijor.aspx?target=ijor:ajrc&volume=14&issue=2&article=010
29	Drug Designing in Discovery Studio	BL Jejurikar, SH Rohane	Asian Journal of Research in Chemistry	https://www.indianjournals.com/ijor.aspx?target=ijor:ajrc&volume=14&issue=2&article=008
30	Role of Aminated derivatives of Natural Gum in Release Modulating Matrix Systems of Losartan Potassium: Optimization of Formulation using Box-Behnken Design	Shankar B. Kalbhare*, Dr. Vivek Kumar Redasani , Mandar J. Bhandwalkar,Rohit K. Pawar , Avinash M. Bhagwat	Asian Journal of Pharmaceutical Research.	https://www.ijpsnonline.com/index.php/ijpsn/article/view/2267
31	A Review: Mechanism And Role Of Superdisintegrants In The Development Of Mouth Dissolving Tablets	Vrushali Ganesh Raut , Pranjal S. Nikam, Bhartee P. Chaudhari, Vivekkumar K. Redasani	International journal of PharmTech Research	https://www.sphinxsai.com/2021/ph_vol14_no1/2/(177-185)V14N1PT.pdf
32	Formulation And Evaluation Of Antifungal Micro emulsion Based Gel For Topical Drug Delivery Using Milletia Pinnata	Bhagyashri Gadave, Atish Velhal and Dr. Vivekkumar Redasani	World Journal of Pharmaceutical Research	https://wjpr.s3.ap-south-1.amazonaws.com/article_issue/da45b45b5ec24c853edb51ce8635ba23.pdf
33	Evaluation of Antiepileptic Activity of Ficusracemosa in Chemicals Induced Epilepsy in Mice	Prajakta J. Chavan *, Atish B. Velhal.Vithal J Chawre, Vivek K. Redasani	Asian Journal of Pharmaceutical Research and Development	https://www.ajprd.com/index.php/journal/article/view/997
34	A Review on Medicinal Plants of Natural Origin for Treatment of Polycystic Ovarian Syndrome (PCOS)	Shivani Andhalkar , Vitthal Chaware, Vivek Redasani	Asian Journal of Pharmaceutical Research and Development	https://www.ajprd.com/index.php/journal/article/view/949
35	Analytical Method Development and Validation of Flecainide Acetate by Chromatographic and Spectrophotometric Techniques	Redasani VK, Agrawal YO, Jagtap MS, Mahajan HS and Surana SJ	Medicinal and Analytical Chemistry International Journal	https://medwinpublishers.com/MACIJ/analytical-method-development-and-validation-of-flecainide-acetate-by-chromatographic-

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36	Role of citrus pectin in biological activity:a review	D.S.kachare, P.K.Ghadge, S.S.Mali	Journal of pharmacovigilance and quality assurance	file:///C:/Users/Admin/Downloads/pectinreviewArticle.pdf

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REVIEW ARTICLE

Review on Guassion, the General Purpose in Computational Chemistry for Medicinal Chemistry

Shubham A. Nangare*, Sachin H Rohane
Department of B Pharmacy at Yashoda Technical Campus, Satara.
*Corresponding Author E-mail: **shubhamnangare251@gmail.com**

ABSTRACT:
In these review we explain all the detailed information about guassian software. Now a days the guassian very much beneficial in to computational chemistry for medicinal chemistry work by the various calculations. This is initially used bye the john poples. Guassiansoftware capable of predicting many properties and calculations of molecules and reaction. Molecular docking also done bye this software. Varios authors wordks on their subject by using this software. I shows interest into guassian because of this is very beneficial for calculations. In guassian varios mathematical equations are added and this will be feneficial or helpful to guide scientist.

KEYWORDS: Molecular docking, Drug Discovery, Guassion Software.

1. INTRODUCTION:

Guassian software is a general purpose computational chemistry software package. Guassian software initially started used in or relesed in 1970 by the scientist john pople. And the scientist john pople started his research group at the Carnegie Mellon University as guassian 70 then this continusly udtaed by them. the name of software originates from scientist pople's use of guassian orbitals to speed up the molecular electronics structure calculation opposed to using slater type of orbital then choice to 9 improve the the performace of the software on computating capacities of current computer hardware for hartee fock calculations. The current updated version of this is guassian 16. This is originally availble through quantum chemistry programme exchnage. it was later licensed out by the university carnegie mellon university ans since 1987 has been developed and licensed by the guassian.

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Guassian:

Original author	**John poples**
Developers	Carnegie mellon university
Initial release	1970, 50 years
Stable release	Guassian 16/2017
Website	Www.guassian.com

We will use the guassian programme in windows environment. Guassian is capable of predicting many properties of molecules and reaction, including the following

- Molecular energies and structures
- Reaction pathway
- NMR properties
- Energies and structures of transition states
- Bond and reaction energies
- Vibrational frequecies
- Molecular orbital
- Atomic charges and electrostatic potential
- Multiple moments

Computation can be carried out on system in gas phase and in their ground state or in an excited state

Guassuian input files:

In this Guassian input files inclufdes several different sections.

- Link 0 commands- locate and name scratch files we will not use this option
- Route section- specify desired calculation type the

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method basic sets and other options
- optional addition sections- additional input needed fo9r specific job type
- Title section- brief description of calculation.

JOB TYPES FOR GUASSIAN INPUT:

There are 3 key components to this specification

1. Job type
2. The method
3. Basic set.

Computer aided drug design:

Simply rational design is the inventive process of finding new medication based on biological target. The drugs are commonly organic small moleculesthats activates or inhibits function of biomolecules such like a protein. That is further give the therapeutic action to the patient. Basic in that isdrug design means the invole in molecules that complementry in shape and size of biomolecules. They bind with each other and form the bond. Drug design not relies on computer modelling. This type of modelling callled as computet aided drug design. Drug design depends on knowledge of 3D structures of biomolecules that is known as structural aided drug design. In addition to small molecules biopharmaceutical includes peptides ans especially therapeutic antibodies are incresingly important class of drug and computational method for improving affinity, selectivity and stability been developed.

Drug design also known as efforts to develop a new drug by molecular modification of lead compound for optimization of desired efferts and minimization of side effects.

Now a days structural based drug design is the growing, interative and powerful approaches includes the structural evaluation of target and drug discovery process it is time consuming and as well as cost cunsuming too developing ideas of new effects and potential drug lead molecule

MOLECULAR DOCKING:

Molecular docking is very useful and intresting beneficial to us docking means the attempt to find best matching between two molecules. Docking is the process in which prdedict the preffere orientation of one ligand when bound in an active site to form stable complex. Aim for the molecular docking is to achive an optimization conformation for both receptor and ligand and the relative orientation between protein and ligand such that free energy of overall system is minimized. successful dockingvmethod search high dimensional spaces effectively and use a scoring function that correctly ranks candidate docking. Importance of the molecular docking is that identification of the ligands, correct binding geometry, prediction of binding affinity. etc. therae are rigid docking is the part of molecular docking in that we studied about internal geometry of receptor and ligand. Another type of docking is flexible docking in that we studied about the enumaration on rotation of the one of the molecules is performed. There are various application of molecular docking like lower free energy structures, calculate differntial binding of ligands, library design, novo design, screening of side effects, specificity of potential drug etc [1-3].

REVIEW OF LITRETURE:

Molecular studies docking charge transfer excitation and wave function analyses valacuclovir a potential antiviral drug this study carried by author Fathima Rizwana and Christina susan abraham and software used is guassian 0.9 [4].

Quantum chemical insight into molecular structure NBO anlysis of hydrogen bonded interaction spectroscopic drg likeness and molecular docking of novel anti covid 19 author for this is SJ. Jenepha Mary and C james study carried by the software guassian 0.9 [5].

Conformational anlysis and quantum descriotors of 2 new imidazole derivative by experimental DFT, AIM molecular docking studies adsorption activity on ghraphene study by author Veena S kumar and MS roxy software used by them is guassian 0.9 [6].

Computational assesment on wave function anlysis molecular conformation and molecular docking explores on 2-5 amino-2methylanilino-4-3 pyridile pyrimidine study by author K arulabraham and S mutha and software is guassian 0.9 [7].

Quantum computational spectroscopic and molecular dockinnn studies on 2acetylthoprene and its bromination deerivatives author is M habib rahman and M raja software is guassian [8].

Synthesis of 1-2-3 bistriazole derivative of embaline and evaluation of its effect on high fat diet streptozotacin induced type 2 diabetes in rats and molecular docking author for this antony stalin and perumol palani guassian 0.9 software used by them [9].

Studies of charged transfer complex og qunodic acid with carboxyllic acid, R kavitha and Biological evaluation mol docking and DFT rajendran are the authors and guassian is software used by them. [10].

Conformational profile vibrational abign NLO properties and moleeular docking of biological active herbicide 1,1 dimethyl phenyleurea K haruna and al Saudi software guassian is used 0.9 [11].

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Detailed quantum mechanical, mol docking QSAR prediction, photovoltaic light homesting efficasy analysis of benzil and its halogenated analogus author Yshyama mary B suresh kumar guassian software used for this. [12].

M abhinaya and etal are the authors for the inhibition of biofilm formation quarnum sensing activity wae done on isolated 3-5-7 tri hydroxy and lave from alstonia scholoris lead by using chemistru guassian 0.9 [13].

New thiazide pyridine and pyrazole derivatives as antioxidant bcandidates synthesis DFT calculations and molecular docking by using guassian softwaew by eatal and yassine kaddouri [14].

2D QSAR and docking study of series gaumerin derivatives as inhibitions of CDR with an applicatiomof molecular docking bu guassian software author is Ranina kasmi and etal [15].

Y shyama and etal do study on the DFT and molecular docking investigation of oxicum derivatives was studied by using guassian [16].

Nasima arshad and etal studied on the structural elucidation DNA binding DFT molecular docking and cytotoxic activity studieson novel design crystal thiosemicarbazides was studied by using guassian software [17].

K haruna and etal bdo study on the confirmational profile and the vibrational assignments NLO properties and molecular docking of biological active herticides 1-1 dimethyl 3- phenykurea studies by guassian software [18].

Y shyamo mary and etal do study on the detailes quantum mechanical molecular docking QSAR prediction photovoltic light havsting efficasy analysis of benzil and its halogenated analogus studies by using guassian 0.9. [19].

Mohammad abdul mumit and tarum kumar studied on DFT studies on vibrational and electron spectro homo lumo, MEP HOMA, NBO and molecular docking analysis of benzyl, hydrazine carbodition by using guassian [20].

Towaeds better modelling drug loading in solid lipid nanoparticles molecular docking experiments done by author Rania hathout, abdelkader a metwally by using guassain software [21].

Spectral investigation, DFT computation and molecular docking studies of the Antimicrobial 5 nitroisatin dimer VK rastogi and VB joyhy are the authors, guassain software used by them. [22].

Investigation of DNA RNA molecules for efficasy and activity of corrosion inhibition by DFT and molecular docking tuzun and C kaya are authors. [23].

CONCLUSION:

The review totally focused on prediction of many properties of molecules and reactions using this software. Guassian software is an computer program helped to chemists, chemical engineers, physicist, biochemist and other scientist to predicting many properties of molecules and reactions. such as energies molecular structures, spectroscopic data ie NMR IR UV etc. These prediction based on present review have been becoming a helpful tool to guide scientist for the prediction of various properties.

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REVIEW ARTICLE

Review on Discovery Studio: An important Tool for Molecular Docking

Shravani S. Pawar*, Sachin H. Rohane
Department of Pharmacy, Yashoda Technical Campus, Wadhe, Satara 415003.
*Corresponding Author E-mail: **shravanipawar118@gmail.com**

ABSTRACT:
In this paper, an overview on discovery studio docking program for analyze and modeling molecular structure, sequence of relevance to life science researcher. This software covers the areas such as ligand design, structure-based design, macromolecule design and engineering, simulations, pharmacophore modeling, quantitative structure activity relationship (QSAR), ADME, predictive toxicity. Discovery Studio help to analyze activities such as anti-convulsant, anti-viral, antidepressant, antibacterial, anti-tubercular, anti-Alzheimer, anti-malarial, anti-cancer. This software gives better result correlation with in-view pharmacological activity. So, this is a helping tool for a researcher to minimize the time as well as cost of research activity and also gives better understanding to study ligand and receptor complex.

KEYWORDS: Drug Discovery, Molecular Docking, Discovery Studio software.

INTRODUCTION:

Computer-Aided drug design represents computational resources that used to facilitate the design and discovery of new therapeutic solutions. Molecular docking is widely used in CADD due to its reliable prediction properties and is one of the essential backbones of CADD. Molecular docking is a rapid and inexpensive technique in academics as well as in industrial settings. Molecular Docking is a technique to find best matching molecular structure by interaction between enzyme and ligand. It also analyses the orientation of one molecule with other into the binding site of a macromolecular target. Docking has a major role in virtual screening, drug discovery, bioremediation.

Discovery studio software is an agglomeration to transcript small molecules and macromolecule system. It is developed by Dassault Systemes BIOVIA (Accelrys). Discovery Studio is a single unified, graphical interface for advanced drug design and protein modeling research. This software provides bunch of viewers for display plots and graphical representation of data.

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Review of literature:

Ana-Maria Udrea and et al use Discovery Studio software to check the potential of phenothiazine in Covid-19 infection. They found that data given by docking software is correlated with in vivo activity. The data found to be suitable and correct with in vivo activity.

Shiben Wang and et al synthesized the different series of 1,3,4-oxadiazole derivatives using discovery studio and the compound were studied gives best anticonvulsant activity. In silico studies were carried out to explore the binding interaction of the most active compound. They found the target compounds were related with in vivo and vitro activity.

Fatma Gur and et al studied the adverse effect of Atomoxetine which issubstitute for methylphenidate in the long-term treatment of ADHD. They conduct molecular docking study using Discovery Studio programs. The data found to be match with in vivo activity.

K. Sangeetha and et al use the discovery studio to check the antiviral activities of plant derived compounds against zika virus. By In silico studies, the software used for screening of various phytochemicals against Zika virus to identify new promising drug candidates. In this study, around 5550 phytochemicals retrieved from

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various databases were subjected for molecular docking in Discovery studio program.

Shi-Ben Wang and et al designed and synthesized the derivatives of coumarin and 3, 4-dihydroquinolinone. They found that compound check in the discovery studio software have best antidepressant activityand also exhibits good affinity for the 5-HT1A receptor. These findings can be useful in the design and synthesis of novel antidepressants.

Fathima Rizwana B and et al studied the molecular docking and binding structures on famciclovir and entecavir compound simulated from Discovery studio program. The geometric structures were optimizedand the band gap energies were calculated using software. Electrostatic Potential (ESP) maps identifies Negative and positive potential regions with help of software. Molecular docking studies confirmed the antiviral activity of the selected compounds.

Geethalakshmi Rajarathinam and et al uses discovery studio for docking of T. decandra with FabZ. They found that isolated flavonoid compound possess excellent anti-P. aeruginosa activity. The in-silico analysis of isolated compound shows possible action in a hypothetical way. The molecular docking of flavonoid was carried out using Discovery Studio.

Sugunadevi Sakkiah and et al developed 3D pharmacophore model based on the known inhibitors. This Pharmacophore model was generated using HYPOGEN algorithm in discovery studio program. From the molecular docking studies around 36 compounds were obtained based on consensus scoring function and selected as HSP90 inhibitors.

The aim of Ran Joo Choi and et al was to evaluate the anti-Alzheimer's disease activities of selected ginsenosides. They use the docking software to check thepotential of ginsenosides in the development of therapeutic agents for Alzheimer's disease. They predict binding energies of the ginsenosides with β-site amyloid and obtained result were corelated with in vitro activity.

Nafees Ahmed and et al use discovery studio for synthesis of tricyclic guanidine derivatives and biological evaluation against P. falciparum. The docking studies show that there is very strong correlation between in silico and in vitro results. Based on the data obtained by software, more potent inhibitor against P. falciparum can be designed. Docking was performed using DS program to understand the mechanism of inhibition and to identify pharmacophore required for anti-malarial activity.

Prashant Bhardwaj and et al uses DS program for identification of novel proteintargets for triclosan. They conduct the inverse virtual screening study for protein targets. A text mining study of triclosan was initially performed to find outinteraction in various biochemical processes.

Amer Hosny and et al use the software for development of a predictive model to identify potential HIV-1 attachment inhibitors. They performed the study in two phased computational process to identify useful compounds capable of binding to the protein for therapeutic purposes using the Discovery Studio docking and screening software.

Mohammad Heiat and et al conduct the study in docking program to analyze isolated ssDNA aptamers against angiotensin II. They found that the structural and sequential homology between aptamers can be considered as a sign of similar characteristics and Output PDB files were modified from RNA to DNA in the discovery studio visualizer software. The in-silico study was performed and uses to find aptameric fragments binding potency.

Sagir Yusuf Ismail and et al performed in-silico QSAR study of sulfur containing shikonin oxime derivatives. The docking study also carried out between this derivatives and target protein. This study provides an approach for the design of more potent anti-colon drug. The data found to be match and correct with anti-cancer agent for colon cancer.

Shola Elijah Adeniji and et al use discovery studio software to molecular docking evaluation of selected quinoline derivatives. Discovery Studio software was used to visualized and analyzed the docked results. They found that activity of quinoline derivatives checked in software exhibits as best anti-tubercular agents.

CONCLUSION:

This study paved better understanding of Discovery Studio software for viewing, sharing, analysing protein and small molecule data. The DS program provides applications covering areas including molecular mechanism, molecular dynamics, quantum mechanics. Software also have ability to perform hybrid QM/MM calculations. It employed for small molecule and macromolecule applications. The molecular properties can found by editing structures and performing calculations.

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REVIEW ARTICLE

Role of Autodock vina in PyRx Molecular Docking

Rutuja P. Pawar*, Sachin H. Rohane
Department of Pharmacy, Yashoda Technical Campus, Satara, 415003.
*Corresponding Author E-mail: **rutujapawar5000@gmail.com**

ABSTRACT:
Molecular docking has been widely employed as a fast and inexpensive technique in past decades, both in academic and industrial setting. In current situation molecular docking software is very useful. It's a need of society. That's why this review is focused on docking. In that Autodock Vena by pyrx version is very useful software for literature. New approaches continue to be developed and value of published work grows at a rapid pace. In resend developments there will be increase in accuracy, time limite, advances in computing power to eventually accomplish the full potential of the area. This review precents the overview of the method and attempt to summerise recent developments regarding four main aspects of molecular docking approaches: bench marking set, Advances in consensus method, recent applications using, use of machine learning, algorithms in docking. This autodock vena software gives more information related to molecular docking by litareture survey.

KEYWORDS: Molecular Docking, Drug Discovery, Autodock vina by PyRx software.

INTRODUCTION:
Doking is on the front line of computational biology and drug discovery the explosion of structural and chemical information in recent years has rendered this use the computation approaches to discover developed and analyzed and similar biologically active molecules the computer aided drug discovery leads to virtual screen, energy calculations, ADME models and drug interactions this helps in scientists in minimizing the synthetic and biological testing.

Autodock vina in PyRx software is most prefferable software in the molecular docking. This software is important. The molecular docking approach can be used to model the interaction between small molecule and protein at the automic level allow as to characterize the behavior of small molecules in the binding site of target proteins as well as elucidate fundamental biochemical process. In drug discovery, protein –ligand or protein – protein. Docking plays an important role in predicting the orientation of the ligand. The ligand is searched in a six dimentional rotational or translational space to fit in the binding site.[1-3]

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REVIEW OF ARTICLES:
Vina design philosophyis not to require the user to understand its implementation. Autodock is suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug conditions bind to recepter of known 3D structure. Autodock vina does not require choosing atom types and pre-calculating grid maps for them. Docking using autodock version 4.0 of PyRx software. PyRx is an open source software to perform virtual screening it is a combination of sevral software.

PyRx includes a docking wizard. Specific aspects for using PyRx as well as consideration for data preparation docking and data analysis also describes. Drug discovery is attractive research area that enables application of cutting edges biomedical research to improve health of man people by active components of natural origin have been under enormous investigation as potential studies that were performed by using PyRx docking tool through autodock vina software M. Venkateshan and Etal used autodock vina PyRx software to check inhibition activity of Azaphenantherena derivatives on or over SARS COV-2. This software to provid the guidance about inhibition activity of Azaphenantherena.[4]

Maryam Fatima et al used PyRx software to check bioactivity and docking of Synthesized ligand. the compound can serve as promising lead for the

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development of new antifungal agents this software is used to check antifungal acivity this compound can serves as promising leads for the development of new antifungal agent.[5]

Usman Abdulfatai et al used to autodock vina verion 4.0 of PyRx software to check activity relationship study of anti-convelsant activity of aminobenzothiazole of their quantitative structure. This software is useful to provide guidance about activity of anti-convalsant.[6]

Usman Abdulfatai et al used autodock vina of PyRx virtual screening software to, molecular docking and quantitative structure activity relationship studies were carried out and 37 anticonvalsant compounds to develop a robust model for the prediction of anticonvalsant activities against gama aminobutyric acid aminotransferase this softare is useful to analyses of a few aminotransferase inhibitor activity.[7]

Ritika Srivastava et al used PyRx software to check a alkylated benzimidazol: designed, Synthesis, Docking, DFT analysis ADMET property activity against HIV and YFV. Series of Alkylated Benzimidazol derivatives was synthesized and screened for their anti-HIV, anti-UFV and broad-spectrum antivirus properties. The software useful to show excellent inhibitory property against the yellow fever virus with.[8]

Muhammad Baba Muh'd et al used PyRx software to study of anti ulcer activity of quinozolinone derivatives of quantitative structure activity relationship by using this software is useful to provide anti ulcer activity. this anti ulcer agents exhibiting good action against the receptor (H/K Atpase).[9]

Titilayo Omolara Johnson et al used autodock vina PyRx software to a ulcerative colitis is an implamation of the colon that can progress colorectal cancer if left untreated no medication completely cures ulcerative colitis and natural products are source of alternative approaches the anti imflamatory potential of phyllanthus nivosus leaf as a natural remady and as a source of new drugs against ulcerative colitis is validated.[10]

Mohammad Abdul Mumit et al used PyRx to study on vibrational and electronicspectra analysis of benzyl-3-N-(2,4,5-trimethoxyphenylmethylene) hydrazine. The absorption, distribution, metabolism, excreation and toxicity investigation predicted that tne compound has good drug like character.[11]

Mohamed Elbadawi et al used PyRx to virtual drugs screening revealing an oxofluorenyl benzamyd and bromonaphthalene sulfonamide hydrobenzoic acid compounds induced apoptosis in a dose dependant manner as analyzed by flow cytomery this two compounds binds to HDAC6 an inhibites its function and exerts cytotoxic activity by apoptosis induction this software used to HDAC6 inhibit.[12]

Shola Elijah Adenji et al used PyRx software to investigating and evaluating some active compounds as potent anti-tubercular agent against MTB CYP121 recepter this. This whole docking result against MTB CYP121 receptor provide a valuable approach for structure based design.[13]

Rina Herowati et al used to PyRx software to studies of chemical constituents of tinos spara cortifolia on glycogen phosphorylase. These software used to give the activity of glycogen phosphorylase in the liver and widely used in the treatment of diabetis mellitus.[14]

Abhay Jaiprakash Gandhi et al used to PyRx software to study the drug for management of SARS-COV2 with ayurvedic perspective along with silico study. The molecular docking and grid were generated this PyRx software of autodock.[15]

Nanda Kumar Yellapu et al used to PyRx software to study the modeling, molecular docking, probing catalytic binding mode of acetyl-CoA malate synthase G in brucella melitensis. The core domain pocket of MSC catalytic residue. these ligand leads could be the best prospective inhibitors to treat brucellosis. These software is useful for activity.[16]

Sabitu Babatunde Olasupo et al used PyRx software is the antidepressant properties in inhibition of serotonin transporter has been considered to be a good target for the treatment of mood disorders. the phenyl piperidine derivatives as inhibitors of serotonin transporter cheminformatics and molecular docking. this software is used to check the antidepressant activity.[17]

Aliyu Wappah Mahmud et al used Autodock vina PyRx software to the quantitative structure activity relationships provides a model that link biological activities of compound to their chemical structures and molecular docking study reveal the interathction between drug and it target enzyme. In this software to check the activity of antiplasmodium hybrid compound.[18]

Adedirin Oluwaseye et al used autodock vina in PyRx software to check on anticonvulsant activity of isoxazole and thiazole derivatives active in animal model. This software useful to provide the information about active in subcutaneous pentylenetetrazole in animal model.[19]

Aliya Nur Hasanah et al used Autodock vina in PyRx software is useful to extraction of atenolol from spiked

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blood serum using a molecularly imprinted polymer sorbent obtained by precipitation polymerization is the Atenolol is cardio- selective B-blocker that is used in the Treatment of hypertension over extended periods. in this software is to check activity of extraction of atenolol fom spiked blood serum in polymerization.[20]

CONCLUSION:

This is an open source software. Autodock vina in PyRx is virtual screening software for computational drug discovery that can be used to libraries of computer against potential drug targets. Using this Autodock vina in PyRx software fordocking the several ligands against one macromolecule. This molecular docking software predicted the activity.

This softeare is useful for the literature. In this software is essential for research of molecular docking. It is inexpensive, time consuming process, it gives accurate result.

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REVIEW ARTICLE

Drug Designing in Discovery Studio

Bhagyashree L. Jejurikar*, Sachin H. Rohane
Department of Pharmacy, Yashoda Technical Campus, Satara 415015 India.
*Corresponding Author E-mail: **bljejurikar@gmail.com**

ABSTRACT:
The process of drug development and drug discovery is very challenging expensive and time consuming. It has been accelerated due to devlopment of computational tools and methods. In current situation lots of molecular docking software are available in the market, during lirerature survey it was found that Discovery Studio is suit of software for stimulating small molecule and macromolecule system. It is developed and distributed by Accelarys. It helps to Investigate and test hypothesis in silico prior it costly experimental implimentation thus reducing the time and expense involved in bringing products to market. it is developed and distributed by Dassault system BIOVIA (formerly Accelerys). During literature survey it was found that Discovery Studio software was efficiently guided to as author regarding mainly biological activities like anti-inflammatory activity, anti-tubercular activity, anti-bacterial activity, anti-viral activity, anti-diabetic activity and anti-oxidant activity.

KEYWORDS: Molecular docking, Drug discovery, Discovery studio.

INTRODUCTION:

Molecular docking is one of the most frequently used methods in structure based drug design, due to its ability to predict the binding- confirmation of the small molecule ligands to the appropriate target binding site. Discovery and development of a new drug is generally known as a very complex process which takes a lots of time and resources. So now a days computer aided drug design are used very widely to increase the efficiency of the discovery and development courses. CADD are evaluated as promising technique according to they need, in between all these structure – based drug design and ligand – based drug design. As very efficient and powerful technique in the drug Discovery and development. These both methods can be applied with molecular docking to the virtual screening for lead identification and optimization. Molecular docking is a key tool in structural molecular biology and computer assisted drug design.

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Discovery studiosoftware is suit for stimulating small molecule and Macromolecule system. The product suite as astrong academic collaboration programme, supporting scientific research and makes use of a number of software algorithms developed originally in the scientific community, including CHARMM, MODELLER, DELPHI, ZDOCK, DMol3 and more [1-3].

REVIEW OF LITERATURE:

Qinggang Meng and et al used Discovery Studio Software check the Rizoma Atractylodis and Rhizoma Atractylodis Macroephalae herbal pairs against type 2 diabetes mellitus. the interaction between targets and ligands were observed and analayzed, according to CDocker interaction energy, most compounds from the herbal pair had good binding activities with receptor and nine compounds had even higher scores than those of the original ligands [4].

Shola Elijah Adeniji and et al used Discovery Studio stimulated Software to check the Anti tubercular modeling, molecular docking stimulation and insight toward computational design of novel compounds as potent antagonist against DNA gyrase receptor. Tuberculosis continue to be critical health problem causing death and illness among millions of people yearly and ranked the second leading cause of mortality

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among the communicable infections in the world. This work accessed the application of modelling technique to predict the inhibitory activity of some prominent compounds which been reported to efficient against mycobacterial tuberculosis [5].

Sabir Ali and et al used Discovery Studio4.0 accelrys Software to check anti bacteriak, anticancer, and molecular docking studies of macrocyclic metal complexes of dihydrazide and diketone. this studies suggest an octahedral geometry for all complexes, compounds found active against B, Substillis and S,aureusand P, aeruginosa and E.coil bacteria, Zn (II) complex showed significant anticancer activity against squamous cell carcinoma cell tested by MTT assay method. Molecular docking studies with EGFR tyrosine kinase were also carried out . all these results show that some of synthesized compound have remarkable antibacterial and anticancer property [6].

Mehmet Gokhan Caglayan and et al used Discovery Studio Software to check the Electrochemical ,Spectroscopic and molecular docking studies of the insteraction between the anti –retroviral Indinavir andds DNA in this study electrochemical and DNA biosensor was developed using a straightforward methodology to investigate the ineraction of indinavir with calf thymus double –stranded deoxyribonucleic acid for the first time. The obtained results can offers insights into the inhibitory activity of indinavir, which could heip to broaden its application, thus indinavir can be used to inhibit other mechanisum and /or hallmarks of viral diseases [7].

Dominic Agyei and et al used discovery studio software 2019 to study physicochemical characterization and drud likeness evaluation of hypotensive peptides encrypted in flaxseed proteome.in this study, hypotensive peptides derived from mature flaxseed protein sequences were predicted in silico using BIOPEP-UWM with nine protease, three each form digestive, plant and microbial sources. In silico prediction of adsorption, digestion, metabolism, excetion and toxicity (ADME/Tox) profile based on physiochemical properties and Lipinski's rule of five showed that the peptides were non toxic and had desirsble drug like properties [8].

Shola Eljiah Adenjiand et al used Discovery Studio stimulated Software to check Quantum modeling molecular and evaluation of some selected quiniline derivative as antitubercular agents: discovery studio visualize software. Ligand - receptor interaction between quinoilne derivatives and the receptor (DNA gyrase). Docking study indicates that compounds 10 of the derivative with promising biological activity have the ulmost binging anti- tubercular drugs with more efficient activities [9].

Nasser Abdulatif Al- Shabib and et al used Discovery studio 4.0 software to investigate the effect of food additive dye "tartrazine" on BLG fibrillation under invitro condition. Molecular docking results ascertained that Tz binds at the hydrophobic cavityand interact with the key amino acid residues involved in the interaction with different ligands, the spectroscopic,microscopic and computational results electrostatic as well as hydrophobic interaction played a very important role in Tz–induced BLG fibrillation under invitro condition [10].

Jian Wang and et al used Discovery Studio Software to study Graphene/Feso4 namocomposite for effective removal of ten triazole fungicides from water solution . Tebucanazole as an example for investigation of the adsorption mechanism by experimental and molecular doking study. The study on adsorption kinetics and thermodynamics were done by taking tebuconazole as an example. Grapheme/Fe_3SO_4 was prepared and utilized as a adsorbent for removal of ten commonly used triazole fungicides in agriculture [11].

Deepu Mathew and et al used Discovery Studio V4.0 Software to check therapeutic molecule for multiple human diseases identified from pigeon pea (Cajanus Cajan L. Millsp) through GC- MS and molecular docking molecular mechanism behind the therapeutic potential of pigeon pea over the human disease such as rheumatoid arthritis, breast cancer, type II diabetes , malaria, measlesand sickle cell disease were revealed through GC-MS identified phyto – compound ligands with candidate protein [12].

Asif Husain and et al used Discovery Studio Software (version4.0,Accelryssoftware) check the molecular docking with COXI and II enzyme, ADMET screening and in vivo anti– inflammatory activity of oxadiazole, thiadiazole and triazole analogus of felbinae. Based on the core structure of felbinac drug, three series (4a-d,5a-d, 6a-n) of five membered heterocylic derivatives containing three heteroatoms were designed and synthesized starting from felbinac. The prepared molecules were the investigated for their anti-inflammatory, ulcerogenicity, and analgesic in experimental animal [13].

Maryam A. Jordaan and et al using Discovery Studio visualizer Software to check virtual screening and DFT calculation of FDA approved compounds similar to the non – nucleoside reverse transciptase inhibitor (NNRTI) efavirenz the compounds were subjected to screening by analyzing their drug likness, such as Lipinski's rule of

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five and ADME properties. Study showed that lovastatin and simvastatin might be considered as lead compounds for further development for COVID -19 main perotease inhibitor [14].

Assia Belhassan and et al used Discovery Studio2016 Software to study novel triazolo- benzodiazepine analogues as antidepressant targeting by molecular docking and ADMET properties prediction. Docking studies suggested that eleven ligands interacted within activesiteof Drosophila melangaster dopamine transporter (Ddat)) (PDBED:4M48) [most ligands formeH-bond with amino acid phe43, Asp46, Asp475, Tyr123, Ser421 and Gln also exhibited Pi and Pi-Pi. In silico ADME evaluation of compounds shoed more than 96% intestinak absortion for all compound [15].

Mohommad k.Parvez and et al used discovery studio software to check plant derived antiviral drugs as novel hepatitis B virus inhibitors: Cell culture and molecular docking study. Docking of lamivudine indicatedstrong interaction with the modeled HBV pol active site residues that formed stable complex, similarly all the docked antiviral compounds formed very stable complexes with anti-HBV pol. Taken together,our data suggest the anti-HBV potential of thetested natural compounds as novel viral pol RT inhibitors [16].

Hanine Hadni and et al used to discovery studio software to check molecular docking andthe antimalarial activity of hybrids 4-anilino-qinoline-triazines derivatives with thewild-type and mutant receptors ofof-DHFR docking studies were performed for previously reported 4-aniloquinoline and 1,3,5-triazines based molecular hybrids. The docking result revealed that these molecular specifically with SER108 and 1LE164 in the pf-DHER binding pocket as that of best active compounds but also showed additional interactions with LEU40 and GLY44 [17].

Aliyu Wappah Mahmud and et al used discovery studio software check QSAR and molecular docking studies of 1,3,dioxoisoindoline-4-aminoquinoline as potent antiplasmodium hybrid compounds. The docking result indicates strong binding between 1,3-dioxosoindoline-4-aminoquinoline and plasmodium falciparum lactate dehydrogenase (PFLDH) ,and revealed the important of the morpholinyl substituent and amide linkerin inhibiting PFLDH. These results could serve as a model for designing novel 1,3-dioxoisoindoline-4-aminoquinolines as inhibitors of PFLDH with higher antiplasmodial activities [18].

K.Jayasheela and et al used discovery studio to check the conformational and spectroscopic characterization, charge analysis and molecular docking profiles of chromone-3-caboxylic acid using a quantum hybrid computational method. The spectroscopic profile of chromone-3-carboxylic acid (abbreviated as 3CA) was exmined using FT-IR, FT-roman,UV,1H and 13C NMR technique. Result of the docking study identified the sugar phosphate inhibitor activity of the target molecular (C3CA) [19].

Mariana Spetea and et al used discovery studio (version3.0) software to check structure activity relationship. Explorations of 14-oxygenated N-Methylmorphonan-6-ones as potent µ-Dpioid receptors against. The crucial role of relative orientation of the ligand in the u or binding site, influencing the property of critical non-covalent interaction that are required to facilitate ligand/-µ or activation by the 14-oxygenated N-methylmorphinan-6-ones,which should be useful for guiding drug design.

Shola Elijiah Adeniji and et al using Discovery Studio Visualizer software. To check the theoretical modeling and molecular docking stimulation for investigating and evaluatiny some active compounds as potent anti tubercular agents against MTB CYP 121 receptor. Docking studies revealed the best molecule with docking scores of–13.7 kcal/mol which formed H- bond and hydrophobic interaction with amino acid residue M. Tuberculosis cytochromes (MTB CYP 121) [20].

Shola Elijiah Adeniji and et al used Discovery Studio Visualizer software check the In silico study for evaluating the binding mode and interaction of 1,2,4-triazole and it's derivatives as potent inhibitors against Lipoate protein B (Lip B) research has shown that the binding affinity of these compounds wery found to be better than the recommended anti–mycobacterium drugs; isoniazid (-14.6kcal/mol) and ethambutol (- 5.8kcal/ mol). This study provide valuable approach for designing and synthesizing more potent anti–mycobacterium tuerculosis derivatives [21].

Ana- Maria Udrea and et al used Discovery Studio Visualizer software to check Laser irradiated phenothiazines: New potential treatment for COVID - 19 explored by molecular docking . In this study predict , using molecular docking, the binding affinity to 15 phenothiazines (antihistaminic and antipsychotic drugs) when interacting with the main protease SARS - coV-2 . Results reveal that thioridazine –and its identified photo products (mesoridazine and sulforidazine) have high biological activity on the virus main protease [22].

CONCLUSION:

This review totally focused on the drug designing in Discovery Studio software and it's different versions such as Discovery Studio V 4.0, Discovery Studio 2016,

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Discovery Studio 4.0 Accelrys. This software also useful to check the various biological activities and therapeutic activities such as Anti -inflammatory, Anti- tubercular, Anti-diabetic and Anti- oxidant activity etc.

After literature review it was seen that number of researchers suggest Discovery studio software for protein – ligand interaction and the interaction between target and ligands were observed and analyzed.

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REVIEW ARTICLE

Organization of Swiss Dock: In study of Computational and Molecular Docking Study

Neha Subhash Patil*, Sachin H. Rohane
Department of B. Pharmacy at Yashoda Technical Campus, Satara.
*Corresponding Author E-mail: **nehap2761@gmail.com**

ABSTRACT:
In the present era, many fields of research are showing great importance. Apart from the applications researchers have grown their interest in pharmaceutical application. Protein play and important side in study of various in - vitro and in - vivo studies to understand the action of drugs. Docking programs have a wide range of applications ranging from protein Engineering to the drug design. Swiss dock software was guide to authors to predict the molecular interactions that may occur between a target protein and a small molecule. After review it was analysed that Swiss dock are organised for UV-VIS spectroscopy, Synthesis, crystal, structures, etc. This article presents Swiss Dock, a web server dedicated to the docking of small molecules on target proteins. It is based on the EADock DSS engine, combined with setup scripts for curating common problems and for preparing both the target protein and the ligand input files. An efficient Ajax/ HTML interface was designed and implemented so that scientists can easily submit dockings and retrieve the predicted complexes. For automated docking tasks, a programmatic SOAP interface has been set up and template programs can be downloaded in Perl, Python and PHP. The web site also provides an access to a database of manually curated complexes, based on the Ligand Protein Database.

KEYWORDS: Molecular Docking, Swiss Dock, Drug Discovery.

INTRODUCTION:
Molecular docking is prediction of the binding affinity To achieve an optimize Conformation for both receptor and ligand and relative orientation between protein and ligand such that the free energy of the overall system minimized Molecular docking is one of the most frequently used methods in structure based drug design, due to its ability to predict the binding conformation of Small molecule ligands to the appropriate target binding site. Molecular docking has become an increasingly important tool for drug discovery Programs based on different algorithm were developed to perform molecular dockings studies which have made docking an increasingly important tool in pharmaceutical research.

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The aim of molecular docking is to give a prediction of the ligand-receptor Complex structure using computation methods Swiss Dock a web Service to predict the molecular interaction that may occur between target protein and a small molecule many binding modes are generated either in a box or in a vicinity of all target cavities [1-3].

Computer aided Drug Design:
Drug design depends on knowledge of 3D structures of biomolecules that is known as structural aided drug design. in addition to small molecules biopharmaceutical induces peptides and especially therapeutic antibodies are increasingly important doss of drug and computational method for improving affinity selectivity and stability been developed.

Most docking programs are Complex computational machineries and require specific additional Sampling or Scaring parameters to which they might be very sensitive while convenient default values are often a proposed good understanding of the user manual and the original

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article is always required in order to achieve meaningful prediction.

Swissdock:

Swiss Dock is the docking web server that addresses limitations described above. Well a the structure of the target protein, as well as that of the ligand, can be automatically prepared for docking. Allcalculation performed on the server side, so that docking runs do not require any Computational power from the user.

A target protein structure can be determined either by specifying its identifier from the protein data Bank. Since the calculation are performed in the CHARMM force field, Swiss dock Supports the uploading of CHARMM. Formatted files in addition to the Commonly used PDB format protein Structure can be uploaded as a set of protein structure file, Coordinate file and extra topology and parameter files if needed. Once the target protein structure has been defined it is immediately prepaid for used with CHARMM, and the Curated structure can be downloaded and reviews prior to the docking assay if needed. The performance of the backend of Swissdock was assessed by a blinding docking assay on 251 test Complexes taken from the ligand protein Database with different presents available from the web interface. The performance of Swiss Dock depends on the number of free dihedrals of the ligand.

Influence of the flexibility of the success rate observed with SwissDock the docking engine of SwissDock redocking assay Carried out on 251 protein ligand Complex.

Table No. 01

Max No. of routable bond of the Ligands	FDA Approved Drug (in %)	SR0 (%)	SR5 (%)
5	63	84	93
10	93	77	86
15	99	69	83
20	100	66	81

The fraction of the surface of the ligand which becomes buried upon complexation also has Significant effect the higher this fraction, the easier it is for Swiss Dock to identify the binding pocket, and therefore to dock the ligand inside.

Table No. 02

Influence of the fraction of the Ligand which is buried upon complexation (% BS) on the same data set.

Min % BS	SR0 (%)	SR5(%)
95	82	95
90	70	85
85	66	80
80	62	75

Swiss Dock Input Files

- Since docking assays are carried out in the CHARMM 22 /27 all-hydrogen force field.
- Target proteins and ligands that have been uploaded as CHARMM- Formatted files Can be used.
- Protein and ligands that have been submitted in PDB or be mold to format respectively have to be Converted prior to the docking itself.

Computer Aided Drug Design:

Simply rational design is the inventive process of finding new medication based on biological target the drugs are commonly organic small moleculesthats activates or inhibits function of biomolecules such like a protein. That is further give the therapeutic action to the patient. Basic in that isdrug design means the invole in molecules that complementry in shape and size of biomolecules. They bind with each other and form the bond. Drug design not relies on computer modelling. this type of modelling callled as computet aided drug design. Drug design depends on knowledge of 3D structures of biomolecules that is known as structural aided drug design. in addition to small molecules biopharmaceutical indudes peptides ans especially therapeutic antibodies are incresingly important dass of drug and computational method for improving affinity selectivity and stability been developed.

Drug design also known as efforts to develop a new drug by molecular modification of lead compound for optimization of desired efferts and minimization of side effects.

- **Web interface Inputs:**

Only three steps are required to start a docking assay through the web interface of SwissDock: users must define a protein structure, one or several putative ligands and docking parameters. They are guided throughout this short and simple submission process by a comprehensive contextual help. As mentioned above, several sample files are supplied to users and can be directly uploaded into the form simply by clicking on a link. The corresponding sample output files are also provided.

- **Target selection:**

A target protein structure can be determined either by specifying its identifier from the Protein Data Bank (15) or by uploading structure files. The first option allows users who are not familiar with 3D structure files to start a docking assay with only a PDB code. If several PDB records are available for the same target, those with a high resolution and a ligand similar to the one that will be docked should be considered first.

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- **Ligand selection:**

A ligand can be selected either by specifying its identifier from the ZINC database (23) or by uploading structure files. The former possibility allows users who are not familiar with 3D structure files to start a docking assay with only a ZINC accession code (AC). The latter allows uploading several ligands at once or uploading ligands that are not present in the ZINC database. As for the target protein, SwissDock not only supports the widely used Mol2 format, but also the direct upload of CHARMM input files describing the ligand.

Review of Litreture:

Sabrin R. M. Ibrahim and Khalid Z. Al-shaliand et al used SwissDock ADME for Molecular Docking studies of the tested metabolites estimated to shade up rational explanation of α – amylase inhibitory activity result the pharmacokinetic parameter [4].

Mohammad Kabrineand et al used SwissDock in article to investigate the possible mechanism by which selected drugs act an silico theoretical molecular docking approach was used, during this study they stimulated the binding mode of N3 against 6lu7 crystal structure using SwissDock to ensure the effectiveness of Dock result and to compare result produced by several drugs to those of N3 [5].

Kerry A. Ramshottom et al used SwissDock programme to investigate that if the software cooled accurately dock the abacavir back into the crystal structure for the protein arising from the known risk allele and if the software is able to distinguish between the HLA-associated and known HLA associated allele [6].

Long Ding and et al used SwissDock software to discover Bioactive peptide silico method peptide would be experimental in-vitro to identify the activity [7].

Dae Hawn-Kim and et al used SwissDock stimulation analysis of unbiased blind docking it was determined the top score predicted blind side for SGI-1027 and M\M to localize the binding region on PrP. The result indicates CHI-1027 interacted with and regions on PrP [8].

Jesus Campagna and et al used SwissDock for evaluation of an Allosteric BACE inhibiter peptide to identify mimetic that can interact with the loop and region of the Enzyme and prevent APP cleavage and to elucidate the mechanism of peptide 65007 allosteric inhibition in silico experiment were performed first by conducting molecular docking in SwissDock with 65007 and comparing the model to crystal structure of the genetech Ab and BACE [9].

Layla K. Mahdi and et al used SwissDock experiment with GlpO model and its Ligands shows surface representation and the carbo-hydrate ligands as sticles. A predicted binding modes for LNT with a surface representation of GlpO model [10].

ElahelKashoni – Amin and et al used SwissDockfor the active site celef is quite extended, some poses were found to occur for the ligand in this location, However a second putative interacting site was found that is located in the entrance of the central beta-barrel of the enzyme. It should be seen that this location was detected by Swiss Dock [11].

Kankana Das and et al used SwissDock in compatible lipid lingad was then docked with proteins that are available as original PDB files were SwissDock interface the results were viewed and analysed [12].

Flavia S. Darquiand et al used SwissDock for putative KpFat A and KpFatB protein structure when moldedbu using homology modling using the Swiss model workspace. Based on their sequence the semi-colum zero-Acpthioesterace crystal structure from G. Californica as a term plate and default primary parametaFurthemore molecular docking was performed [13].

Christina E. Smith and et alused SwissDock for performing the docking of NSAIDs with Capase-3 was performed [14].

Jamal Quazzaniand et al used SwissDock in Silico studies for the docking of GP269, target was prepared from the X-ray protein structure of the crystalized complex. Polar hydrogen atom were added to the protein, The docking of GP269 into the structure of Tb6PGL was performed using the Swiss Dock [15].

CONCLUSION:

From Reviewing the above literatures, it was found that the Swiss Dock Software is used to predict the molecular interaction that may occur between a target protein and a small molecule.

The Swiss Dock web server aims at providing a wide scientific community with a free and user-friendly, yet stateof-the-art protein/small molecule, docking tool. The automatic setup of protein and ligand structures, the different parameter presents and the convenient visualization and analysis of docking predictions makes it accessible to a wide audience. The EADock DSS engine behind SwissDock is especially suited for drug design, with very good success rates for small and relatively rigid ligands with less than 10 flexible rotatable bonds: the most favourable predicted BM is

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found within 2 A˚ to the crystal structures for 77% of the 251 test complexes, and for 86% of them, such a correct BM is found within the five most favourable ones. This performance is even increased if the ligand can be buried in a well-defined binding site of its target protein.

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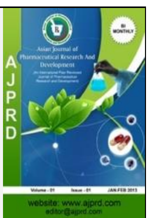

Research Article

Acute Toxicity Study and Anti-Nociceptive Activity of Ethanol Extract of *Aesculus Indica* Seeds on Experimental Animal Models

Priyanka R. More*, Atish B. Velhal, Vitthal J. Chaware, Vivek Kumar K. Redasani

YSPM's Yashoda Technical Campus, Wadhe, Satara, MS, India.

ABSTRACT

Aesculus indica, widely known as the horse chestnut tree, has long been used as antiangiogenic, antibacterial, antidiabetic, antiviral and antifungal. Ttraditionally it has been used as medicine for the treatment of skin diseases, rheumatism and different pain conditions. The current study was undertaken to investigate possible effects of ethanol extract of seeds of plant in experimentally produced pain in animals because there were no scientific publications on the use of *Aesculus indica* seeds for anti-nociceptive activity. Preliminary phytochemical screening revealed the presence of flavonoids, tannins, carbohydrates, Saponins, and phenolic substances in the extract. The OECD guideline 423 was followed for acute toxicity testing.At a dose of 2000 mg/kg, the extract was confirmed to be safe. The anti-nociceptive effect of three distinct dose levels of extract (100, 200, and 300 mg/kg) was tested in Swiss albino mice using a hot plate, tail immersion test, and acetic acid induced writhing. Extract had strong anti-nociceptive efficacy (P < 0.001) in a hot plate test. The extract significantly increased the tail withdrawal reaction in the tail immersion test (P < 0.001).The extract considerably reduced the number of writhes in the acetic acid writhing test (P < 0.001). The findings indicate that the extract has substantial anti-nociceptive effect.

Key Words-Anti-nociceptive, Acute toxicity, *Aesculus indica*, Phytochemical screening.

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***Address for Correspondence:**

Priyanka R. More ,YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara, MS, India.

INTRODUCTION

Pain is more than just a vexing sensation; it is a complicated sensory mechanism. Pain is caused by the activation of nociceptors at peripheral nerve terminals in response to tissue injury, which results in the release of a range of chemicals that change the local environment and cause pain.[1] Many pathological aches progress due to inflammatory responses in the central and peripheral nerve systems.[2] When nociceptive signals from the peripheral nerve system to the brain are regulated, pain is usually felt. In reaction to tissue injury or stimuli, a variety of chemicals such as histamine, bradykinin, and prostaglandins are released, resulting in nociception.[3]Also implicated in nociception are endogenous opioid and cannabinoid receptors[1]. Treatment of pain in chronic inflammatory disorders such as rheumatoid arthritis is a major issue for clinicians and the general public, as chronic use of current synthetic medications has dangerous side effects that cannot be ruled out. This needs the creation of a new nociceptive agent that is both safe and effective in eradicating or minimising unwanted effects. Herbal medications are used in several developing countries, despite the fact that they are not documented in science. Traditional folk remedies are used by about 80% of the population in some developing nations.[4]Pain has traditionally been treated using a variety of herbal medicines. Phytoconstituents have been scientifically verified for anti-nociceptive action.[5-8]The seeds of *Aesculus indica* (Family –Sapindaceae) arerich in saponins most specifically aescin.[9] Aescin has been reported to have anti-nociceptive activity via inhibition of oxidative stress and inhibition of prostaglandins.[10] Additionally with saponins*Aesculus indica*also contains flavonoids and tannins it may show anti-nociceptive activity. Moreover Anti-nociceptive activity forextract of *Aesculus indica* leaves has been reported in the literature.[11] Hence the

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present research work was aimed to evaluate anti-nociceptive activity for ethanol extract of *Aesculus indica* seeds in experimental animal models.

MATERIALS AND METHODS

Drugs and reagents

Aspirin was purchased from USV Pharma, Mumbai, India and pentazocine was purchased from Themis medicare Ltd., Haridwar, India. All chemicals and reagents used for the experiments were of analytical grades.

Collection and authentication of plant material

Seeds of the *Aesculus indica* were procured from Royal Rifco Company, Shrinagar, India.After collection seeds were cleaned, washed to remove any dirt, dust and foreign particles. Botanical identity of plant specimen was authenticated by Dr. S. A. Mohite,Head, Department of Botany, Lal Bahadur Shastri College, Satara (MS), India.A voucher specimen of theseeds has been deposited in the department for future reference.Theseeds were coarsely powdered and further utilized for preparation ofethanol extract.

Preparation of ethanol extract

The ethanol extraction of seeds of *Aesculus indica* was carried out by soxhlet apparatus. The seeds were crushed and ground to powder and placed into extractor. The ethanol was poured on powder with three cycles. After that extraction process was started and continued till appearance of solvent in syphon tube turns brown to clear. Then brown colored solvent mixture from round bottom flask was collected and evaporated with the help of rotary evaporator to get a solid residue. The residue was placed in a vacuum desiccator and was further used for the experiments.

Prelminary phytochemical screening:

Prepared ethanol extract was subjected to preliminary phytochemical screening for presence of Alkaloids, Glycosides, Carbohydrates, Phenolic compounds, Flavonoids, Saponins, Reducing sugars.[12,13]

Experimental animals

Swiss albino mice (18–25 g) were provided byYashoda Technical Campus, Faculty of Pharmacy, Wadhe. Satara. Animals were housed and maintained according to standard guideline and procedures with animal facility at relative humidity 75 ± 5% temperature 22 ± 2° C, and a 12 h light/dark cycle. Animals were provided with standard diet and purified water ad libitum. Mice were allowed to acclimatize to the environment for seven days before start of the animal study. The experimental protocol was approved by Institutional Animal Ethics Committee. All the animal experimental procedures were performed according to the National Institutes of Health (NIH) guidelines on handling of experimental animals.

Acute oral toxicity study

The acute oral toxicity was performed as per the Organization for economic co-operation and development (OECD) guideline 423.[14]Acute toxicity study was performed in Swiss albino mice. The animals were grouped with three numbers in each. Ethanol extract of *Aesculus indica* seeds was given to animals with starting dose 300mg/kg in 0.1% CMC for first group.

According to observations of first group, study was carried out further on next group with dose 2000 mg/kg. From obtained results it was clear that no death as well as no toxicological signs in animals so, for confirmation of safety of extract study was repeated with dose 2000mg/kg on third group. After administration of extract, animals were observed carefully for first 30 min. and periodically for 24 h with special attention during first four hours. Animals were further observed daily for subsequent 14 days. Effects such as changes in skin fur, eyes and mucous membranes were observed daily. Also the circulatory, autonomic, respiratory, and central nervous systems, behaviour pattern and somatomotor activities were observed during study. Animals were further observed for salivation, diarrhea, tremors, lethargy, convulsions, sleep, and coma. The parameters like body weight, food, and water intake were checked periodically every two days.

Anti-nociceptive activity:

The anti-nociceptive activity of ethanol extract was tested using different animal models namely hot plate, acetic acid induced writhing and tail immersion test. Doses of extract were selected based on results of acute toxicity study.

Healthy Swiss albino mice (18–22 g) were used for the study. Animals were divided into five groups of six in each. Group I was control and received 0.1% CMC, group II was received standard drug, group III, IV and V were received ethanol extract of *Aesculus indica* seeds with low, medium and high dose by oral route.

Evaluation parameters

Tests were performed on same animals after 14 days washing period.

Hot plate test

Analgesic activity in mice was executed according to the method described previously.[15,16] The hot plate analgesiometer (IITC, USA) was used to determine the analgesic activity of ethanol extract of *Aesculus indica* seeds. Animals were divided into five groups of six in each. Group I received vehicle i.e 0.1% CMC by oral route, group II received standard drug pentazocine 17 mg/kg by intraperitoneal route, group III, IV and V were received ethanol extract of *Aesculus indica* seeds 100mg/kg, 200mg/kg and 300mg/kg respectively in 0.1% CMC solution by oral route.

Animals were placed on hot plate at different time points (0, 15, 30, 45, 60, 90 and 120 min) after administration of standard drug pentazocin, extract and vehicle, time require for first response (flickering or licking of hind paw or jumping) has been measured. Hot plate was maintained at 55 ± 0.5 °C and cutoff time fixed for 15 sec. to avoid tissue damage.

Acetic acid induced writhing test

The acetic acid induced writhing test was performed as described previously using 0.1 ml of 0.6% v/v acetic acid solution in normal saline.[17,18] Swiss albino mice (18–22 g) of either sex were divided into five groups of six in each.

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Group I received vehicle i.e 0.1% CMC by oral route, group II received standard drug Aspirin 30 mg/kg by oral route, group III, IV and V received ethanol extract of *Aesculus indica* seeds 100mg/kg, 200mg/kg and 300mg/kg respectively in 0.1% CMC solution by oral route. Thirty minutes after administration of standard drug Aspirin and test extracts, 0.1 ml of 0.6% acetic acid were administer via intra-peritoneal route. The number of writhing will be counted for 20 minutes after administration of acetic acid. The percentage inhibition of writhing has been calculated.

percentage inhibition of writhing = (C – T / T) x 100

Where C- Average number of writhes in control group.

T- Average number of writhes in test group.

Tail immersion test

The tail immersion test was carried out in Swiss albino mice (18–22 g) according to method described by previous researchers.[19,20] Animals were divided into five groups of six in each. Group I received vehicle i.e 0.1% CMC by oral route, group II received standard drug pentazocine 17 mg/kg by intraperitoneal route, group III, IV and V were received ethanol extract of *Aesculus indica* seeds 100mg/kg, 200mg/kg and 300mg/kg respectively in 0.1% CMC solution by oral route. Animals were adapted for restrainer 30 min. before study leaving the tail hanging out freely. After administration of standard drug pentazocine, extract and vehicle, the tail immersed in hot water (Temperature 55 ± 0.5° C) and the reaction time require for removal of tail has been recorded as response. The cutoff time for tail exposure to hot water fixed to 15 s. The response was recorded at 0, 15,30,60,90,120 and 180 minutes after administration of dose.

Statistical Analysis

The data presented as a mean ± SD (Standard Deviation). Two way ANalysis Of VAriance (ANOVA) was used to make comparisons between the treated groups. The level of statistical significance was set at $P < 0.001$.

RESULTS

Preliminary phytochemical analysis:

Table 1.Shows the findings of qualitative analysis of *Aesculus indica* seeds extract. According to the obtained resultscarbohydrates, saponins, tannins, flavonoids were found to be present in extract.Alkaloids, glycosides, amino acids, steroids and terpenoids were found to be absent.

Table 1. Qualitative analysis of the phytochemicals inseeds extracts of *Aesculus indica*.

Sr. No.	Test for Phytoconstituents	Present/Absent
1.	**Alkaloids**	
	Mayer's Test	Absent
	Dragendroff's Test	Absent
	Wagner's Test	Absent
	Hager's Test	Absent
2.	**Glycosides**	
	Keller Killiani's test (Cardiac Glycosides)	Absent
	Borntrager's test (Anthraquinone Glycosides)	Absent
3.	**Carbohydrates**	
	Molish Test	Present
	Fehling test (reducing sugar)	Present
4.	**Steroids**	
	Salkowski's Test	Absent
5.	**Flavonoids**	
	Lead Acetate Test	Present
	Sodium Hydroxide Test	Present
6.	**Saponins**	
	Foam Test	Present
7.	**Tannins and phenolic compounds**	
	Ferric Chloride Test	Present
	Lead Acetate Test	Present
	Dilute Nitric Acid Test	Present
	Dilute Iodine Solution Test	Present
	Acetic Acid Solution test	Present
8.	**Proteins**	
	Biuret test	Absent
9.	**Amino acids**	
	Ninhydrin Test	Absent

Acute toxicity study

The acute toxicity study began with a 300mg/kg starting dose. During a 14-day observation period, oral administration of a 300 mg/kg dosage of ethanol extract of *Aesculus indica* seeds caused no significant toxicity. From above results it is clear that given dose was safe and hence further study was performed by administering 2000mg/kg dose of extract to next group of animals. There were no indicators of toxicity and mortality [Table 2.], as well as the animals' morphological characteristics and general appearance did not change. There was no salivation, diarrhoea, tremors, convulsions, lethargy or unusual behavior observed during study in treatment group. When compared to control group mice, extract-treated animals did not demonstrate any significant changes in body weight, food and water intake [Table 4.]. For further confirmation of results effect was checked by giving same dose (2000mg/kg) to another group of three animals and results

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were repeatedly same. Table 3, shows the parameters measured before and after the test extract of *Aesculus indica* seeds. According to results even at the highest dosage of 2000mg/kg body weight of the test animal, all parameters were normal. The oral LD_{50}could be over 2000mg/kg body weight. As a result, greater dose testing of the extracts may not be necessary, and the extracts were practically non-toxic.

Table 2. Effect of *Aesculus indica* seeds extract for sign of toxicity and mortality (n = 3).

Group	Treatment	Sign of toxicity (ST/NB)	Mortality (D/S)
Normal Control	Vehicle	0/3	0/3
Aqueous extract	2000 mg/kg	0/3	0/3
Alcoholic extract	2000 mg/kg	0/3	0/3

ST = Sign of toxicity, NB = Normal behaviour, D = Died, S = Survived.

Table 3. Effects of A.i. seeds extractat dose 2000mg/kg on morphological characteristics and general appearance in mice (n=3).

Sr. No.	Response	Before	After
1.	Alertness	Normal	Normal
2.	Touch response	Normal	Normal
3.	Torch response	Normal	Normal
4.	salivation,	Normal	Normal
5.	Diarrhoea	Absent	Absent
6.	Tremors	Absent	Absent
7.	Convulsions	Absent	Absent
8.	Lethargy	Absent	Absent
9.	Skin fur	Normal	Normal
10.	Pinna reflux	Normal	Normal
11.	Corneal reflux	Present	Present
12.	Pupils	Normal	Normal
13.	Lacrimation	Normal	Normal
14.	Gripping strength	Normal	Normal
15.	Urination	Normal	Normal
16.	Hyper activity	Absent	Absent

Table 4. Effect of extract of *Aesculus indica* seeds extract 2000mg/kg on body weight, food intake and water intake of mice (n = 3).

Day	Normal control			Test group		
	Body weight (g)	Food intake (g)	Water Intake (ml)	Body weight (g)	Food intake (g)	Water intake (ml)
0	19.20 ± 1.15	6.16 ± 0.51	6.86 ± 0.25	19.60 ± 0.85	6.06 ± 0.23	6.20 ± 0.17
2	19.23 ± 1.05	6.20 ± 0.36	6.93 ± 0.73	19.73 ± 0.90	6.20 ± 0.20	6.66 ± 0.40
4	19.56 ± 0.95	6.06 ± 0.61	7.13 ± 0.72	19.80 ± 0.80	5.90 ± 0.17	6.90 ± 0.10
6	19.76 ± 0.76	6.20 ± 0.36	7.20 ± 0.65	20.03 ± 0.85	5.83 ± 0.05	7.03 ± 0.56
8	19.90 ± 0.75	6.26 ± 0.25	6.93 ± 0.51	20.20 ± 0.90	6.16 ± 0.15	7.03 ±0.11
10	20.20 ± 0.65	6.46 ± 0.41	7.30 ± 0.45	20.50 ± 0.75	6.33 ± 0.15	7.03 ±0.23
12	20.46 ± 0.65	6.46 ± 0.57	7.03 ± 0.11	20.56 ± 0.86	6.03 ± 0.20	6.76 ±0.25
14	20.66 ± 0.60	6.46 ± 0.41	7.00 ± 0.17	20.93 ± 0.75	6.13 ± 0.30	6.80 ±0.30

All data is expressed as Mean ± SD (n = 3).

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Antinociceptive Activity:

Hot plate test:

The effect of ethanol extract of *Aesculus indica*seeds is represented in Table 5. Extract significantly delayed the response dose dependently at doses 100, 200 and 300 mg/kg between 15 and 120 min after administration of extract when compared to control group. At dose 300 mg/kg showed maximum response at 45 min with response time 8.80 ± 0.14 ($p < 0.001$) when compared with control group (3.05 ± 0.18). The standard drug pentazocine showed maximum response at 30 min with reaction time 10.03 ± 0.17 ($p < 0.001$) when compared with normal control animals (3.2 ± 0.30).

Table 5: Effect of ethanol extract of *Aesculus indica* seeds in hot plate method.

Groups	Reaction time in seconds						
	Basal	**15 min.**	**30 min.**	**45 min.**	**60 min.**	**90 min.**	**120 min.**
Normal control	2.88 ± 0.14	3.06 ± 0.19	3.2 ± 0.25	3.05 ± 0.18	2.96 ± 0.08	3.03 ± 0.10	3.00 ± 0.15
Pentazocine 17 mg/kg i.p.	2.93 ± 0.26***	8.70 ± 0.08***	10.03 ± 0.16***	9.93 ± 0.17***	7.80 ± 0.14***	5.78 ± 0.07***	4.76 ± 0.12***
Ai extract 100mg/kg p.o.	2.86 ± 0.10***	4.01 ± 0.14***	6.18 ± 0.17***	6.58 ± 0.17***	4.83 ± 0.17***	3.88 ± 0.14***	3.25 ± 0.15
Ai extract 200mg/kg p.o.	2.68 ± 0.14***	5.31 ± 0.14***	6.68 ± 0.19***	7.21 ± 0.19***	5.03 ± 0.12***	4.38 ± 0.28***	3.53 ± 0.32***
Ai extract 300mg/kg p.o.	2.98 ± 0.14***	6.11 ± 0.25***	7.36 ± 0.16***	8.80 ± 0.14***	7.66 ± 0.20***	5.08 ± 0.22***	4.63 ± 0.08***

All data is expressed as Mean ± SD (n = 6).

*** $p < 0.001$when compared with control.

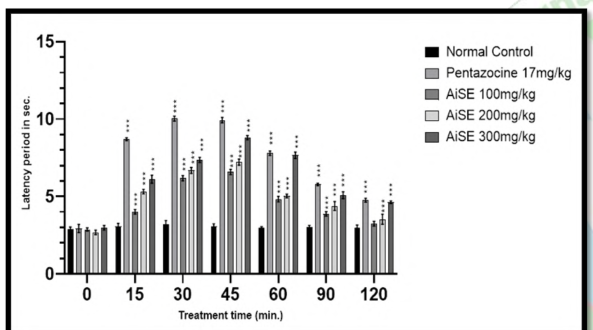

Figure 1: Effect of the *Aesculus indica* seeds extract and pentazocine on the latency time of mice in hot plate model. Values shown are mean ± SD, *** $p < 0.001$when compared with control, n=6.

Tail immersion test

The table 6. Shows effect of ethanol extract of *Aesculus indica* on time required for tail withdrawal response in mice. Extract at all doses significantly increased response time in comparison with control group. The highest dose (300 mg/kg) of extracts (9.63 ± 0.23; $p < 0.001$) showed maximum response at 1.5 h which was considerable response in comparison with pentazocine (17 mg/kg) (12.55 ± 0.25).

Table 6: Effect of ethanol extract of *Aesculus indica* seeds in tail immersion test.

Groups	Time for tail withdrawal response (Seconds)						
	Basal	**15 min.**	**30 min.**	**60 min.**	**90 min.**	**120 min.**	**180 min.**
Normal control	2.11 ± 0.23	2.06 ± 0.10	2.25 ± 0.13	2.18 ± 0.17	2.21 ± 0.18***	2.30 ± 0.16	2.25 ± 0.19
Pentazocine 17 mg/kg i.p.	2.15 ± 0.17***	4.08 ± 0.16***	7.05 ± 0.16***	10.10 ± 0.19***	12.55 ± 0.16***	10.08 ± 0.16***	8.21 ± 0.13***
Ai extract 100mg/kg p.o.	2.18 ± 0.11***	3.05 ± 0.12***	4.60 ± 0.26***	6.13 ± 0.13***	7.30 ± 0.14***	4.26 ± 0.18***	3.25 ± 0.13***
Ai extract 200mg/kg p.o.	2.21 ± 0.11***	3.26 ± 0.19***	5.25 ± 0.22***	7.35 ± 0.13***	8.36 ± 0.11***	6.16 ± 0.08***	4.16 ± 0.17***
Ai extract 300mg/kg p.o.	2.11 ± 0.13***	3.53 ± 0.12***	6.21 ± 0.11***	8.26 ± 0.10***	9.63 ± 0.16***	7.53 ± 0.17***	5.25 ± 0.10***

All data is expressed as Mean ± SD (n = 6).

*** $p < 0.001$when compared with control.

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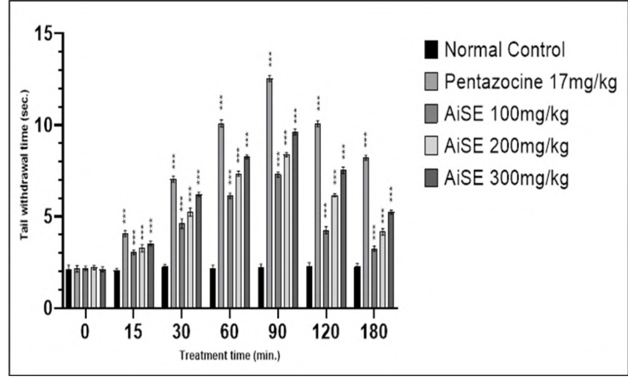

Figure 2: Effect of the *Aesculus indica* seeds extract and pentazocine on the reaction time of mice in Tail immersion model. Values shown are mean ± SD,*** $p < 0.001$when compared with control, n = 6.

Acetic acid induced writhing test

Table 7.Shows the effect ofethanol extract of *Aesculus indica* seeds on the number of writhing in mice. When compared to the control group, extract significantly reduced the number of writhes at doses of 100 mg/kg, 200 mg/kg, and 300 mg/kg. In comparison with control group (44.66 ± 1.44) the extract at 300 mg/kg showed the greatest suppression of writhes (27.16 ± 1.16, $p < 0.001$). When compared to the normal control, the aspirin (30 mg/kg) inhibited writhing by 87.41 percent, whereas the extract at 300 mg/kg inhibited writhing by 64.43 percent.

Table 7: Effect of ethanol extract of *Aesculus indica* seeds in writhing test.

Groups	No. of writhing	% Inhibition
Normal control	44.66 ± 0.81	-
Aspirin 30 mg/kg p.o.	23.83 ± 0.75***	87.41
AiSE 100mg/kg p.o.	37.60 ± 0.81***	18.77
AiSE 200mg/kg p.o.	32.5 ± 0.54***	37.41
AiSE 300mg/kg p.o.	27.16 ± 0.75***	64.43

All data is expressed as Mean ± SD (n = 6).

*** $p < 0.001$when compared with control.

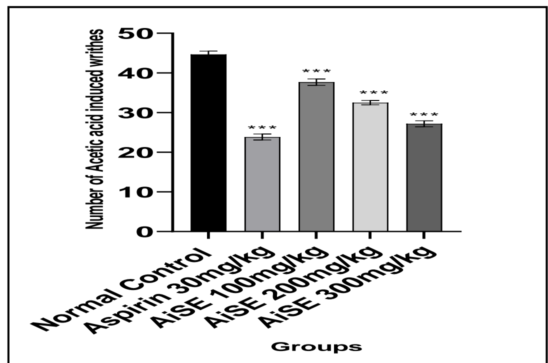

Figure 3. Inhibitory efect of the *Aesculus indica* seeds extract and aspirin on the acetic acid-induced writhes in mice, Values shown are mean ± SD, *** $p < 0.001$when compared with control, n = 6.

DISCUSSION

For centuries, medicinal plants have been utilised to treat human illnesses. The active compounds are mostly responsible for the crude drug's biological activity. However, evidence-based scientific research on the biological activity and toxicity of medicinal herbs are limited. The findings of this study revealed that the *Aesculus indica* seeds extract contained carbohydrates, saponins, tannins, and flavonoids [Tab.1]. These secondary metabolites produce a definite physiological action on the human body.[21-27] Toxicity data aids in determining the maximum dose of a substance that can be safely utilised in animals and humans. There were no reports on the toxicity of *Aesculus indica* seeds extract. As a result, the current investigation began with acute toxicity of the extract at a dose of 300 mg/kg and then progressed to a dose of 2000 mg/kg after obtaining satisfactory results. During 14 days of treatment with a single dose of *Aesculus indica*, there was no death. There was no substantial change in body weight, food, or drink intake, safety of extract.No symptoms of toxicity were noticed at the limit dose throughout the investigation, indicating that it was well tolerated. The findings indicated that the extract is safe to use.

The central mechanism of analgesic action of the extract was evaluated using hot plate and tail immersion techniques.To measure central anti-nociceptive activity, the tail immersion and hot plate tests are standard and very sensitive assays. With this nociception models, the extract showed substantial action. It is well known that centrally acting analgesics raise the pain threshold of mice when they are exposed to heat.[28] These tests are important in determining whether or not a heat induced nociception is present and whether or not narcotics are involved.[29] The hot plate test is very sensitive test to determine central anti-nociceptive activity. It involve neuronal signaling pathways to respond thermal stimuli.Supraspinal reflex is elicited by hot plate method. The substances, which increases the reaction time against heat stimulus, act centraly to mimic pain.[30]*Aesculus indica* (100, 200 and 300 mg/Kg) prolonged the latency period in hot plate model and tail withdrawing time as that produced by pentazocine, a standard analgesic drug indicating the centraly mediated anti-nociceptive activity.

Both tests use neural signalling pathways to respond to heat stimuli and are linked to central activity.[31] As a result, these tests were used to distinguish between central and peripheral analgesics. The hot plate involves higher brain functions and indicates a supra-spinally structured reaction to thermal pain stimuli, whereas the tail immersion test involves spinal motor reflexes to thermal nociceptive stimuli. The effect of central analgesics like opioids is mediated via regulation of spinal (μ2, κ1, δ2) and supraspinal (μ1, κ3, δ1, σ2) receptors.[32]

To test peripheral nociception acetic acid-induced writhing paradigm was used. Acetic acid causes the hind limbs to extend, the back to arch, and the abdominal muscles to contract in response to peripheral nociception.[33,34] TNF-, interleukins, and other inflammatory mediators such as histamine, bradykinin, serotonin, substance P and prostaglandins are released when acetic acid is given intraperitoneally.[35,36]The nociceptors in the dorsal horn of the central nervous system are activated by cytokines and inflammatory mediators, which then activate inflammatory

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pathways, resulting in pain feeling.37,38 These mediators stimulate chemosensitve nociception, developing abdominal constrictions. Such pain sensations are inhibited by non-steroidal anti-inflammatory drugs (NSAIDs), which exhibit antinociceptive effect by inhibiton of prostaglandin synthesis.[39,40]Ethanol extract*of Aesculus indica* seeds reduced the acetic acid-induced writhing, similar to caused that of aspirin revealing the antinociceptic effect of *Aesculus indica*, possibly through the inhibition of peripheral pain mediated pathways.

The presence of saponins, flavonoids and tanins in *Aesculus indica* may account for the anti-nociceptive effect found, as these phytochemicals are renowned for their analgesic effect, while the involvement of additional constituents present in the plant cannot be overlooked.

CONCLUSION

The current study found that at the dose levels tested as per the acute toxicity studies, the ethanol extract of *Aesculus indica* seeds has considerable dose dependent antinociceptive effects in laboratory animals. The findings show that antinociceptive activity of *Aesculus indica* is mediated by two analgesic pathways: peripheral and central.

The tail immersion and hot plate tests are linked with central activity and involve neuronal signaling pathways to respond thermal stimuli. Tail immersion test involves spinal motor reflexes to thermal nociceptive stimuli, whereas the hot plate involves higher brain functions and represents supra-spinally organized response to thermal pain stimuli. The peripheral nociception induced by acetic acid was inhibited by extract. Possible mechanism peripheral analgesic effect may likely due to cytokine inhibition and other inflammatory mediators.

The presence of saponins, flavonoids and tannins in *Aesculus indica* seeds, along with the existence of other phytochemicals, may be responsible for antinociceptive effects. The findings appear to back up the plant's historic use in the treatment of several painful illnesses and also point to the presence of biologically active compounds. However more research is needed to isolate and characterize the bioactive components responsible for the anti-nociceptive effect.

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A Review: Mechanism and Role of Superdisintegrants in the Development of Mouth Dissolving Tablets

Vrushali G. Raut[1], Pranjal S. Nikam[2], Bharatee P. Chaudhari[3], Vivekkumar K. Redasani[4]

[1,2] Department of Pharmaceutics,
YSPM'S Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara 415011, Maharashtra, India

[3]Assistant Professor
YSPM'S Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara 415011, Maharashtra, India

[4]Principal
YSPM'S Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara 415011, Maharashtra, India

Abstract : Because of their ease of administration and patient compliance, mouth dissolving tablets have become more common among strong dosage types. They outperform traditional tablets in terms of efficiency. It aids in the enhancement of oral bioavailability. Waterless administration and quick onset of operation are two major advantages of mouth dissolving tablets. For any solid dosage type, disintegration is a critical phase. Superdisintegrants are a class of younger agents that have been produced in recent years. Superdisintegrants come in a variety of forms, including normal, synthetic, and co-processed. The aim of this article is to discuss the different types of superdisintegrants and their mechanisms in mouth dissolving tablets.

Index Terms -Disintegration, Superdisintegrants, Mouth dissolving, Classification, Mechanism.

1.Introduction:

In an aqueous atmosphere, superdisintegrants are agents applied to tablet and certain encapsulated formulations to facilitate the breakdown of tablet and capsule "slugs" into smaller fragments, thus expanding the available surface area and facilitating a more rapid release of the medication material. They help the tablet matrix to absorb moisture and disperse.[1-3]

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Mouth dissolving tablets are new medication delivery devices with fast disintegration capabilities that have recently gained prominence by addressing the drawbacks of traditional tablets. It is a solid unit dosage type containing active agent that disintegrates rapidly as it comes into contact with saliva without the use of water or chewing.[4]Disintegration is a crucial stage in the operation of any solid unit dosage type, such as tablets or capsules. Disintegrating agents are used in the solid dose formulations in this case. Fast disintegration is essential for quicker drug release and action in mouth dissolving tablets, so superdisintegrants are added to help with faster disintegration. They're used at a lower concentration of 1-10% by weight of the overall weight of the dosage units.[5] Different forms of superdisintegrants are available, and they are used in mouth dissolving tablet formulations depending on their source and method of action. Tablet disintegration is influenced by a number of superdisintegrant causes, including.[6]

Percentage of disintegrants present in the formulation.

a) Proportion of superdisintegrants used.
b) Compatibility with other excipients.
c) Method of addition of superdisintegrant.
d) Presence of surfactants.
e) Nature of drug substance added.
f) Hardness of the tablets.
g) Method of mixing of addition.[7,8]

Because disintegration is so important in tablet dissolution before the active drug substance is finally released from the tablet structure into the body, disintegrant properties (e.g., disintegration time [DT] and the ratio of crushing strength-friability to disintegration time [CSFR/DT]) are influenced to a large extent by the type, concentration, and efficiency of disintegrants.[9]

Advantages of superdisintegrant:

- Should be seen at low concentrations.
- Less focus is needed.
- Intragranularly, it's more powerful.
- It is biodegradable.
- Wetting has a remarkable ability to cause accelerated disintegration.
- There are no lumps formed during disintegration.
- It's safe to use with common medicinal agents and excipients.
- Has a lower impact on compressibility and flow capacity so it doesn't cling to the punches and dyes.
- Some are anionic, and cationic drugs can induce some in vitro binding.[10,11,12]

Disadvantages of Superdisintegrants:

- More susceptible and hygroscopic in nature;
- Moisture sensitivity causes instability;
- Expensive.
- It's time-consuming and delicate.[13]

Ideal properties of superdisintegrants:

- It can disintegrate quickly, have a low water solubility, and have excellent moulding and flow properties.
- The particle size, hydration power, and compressibility index should all be fine.
- It should be compatible with the other excipients and have tableting properties that are desirable.
- It does not form complexes with the medications, be nontoxic, and have a pleasant mouth feel.
- Effective at low concentrations and can disintegrate more efficiently.
- The tablets should be compactable and less friable.[12,14,15,16]

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Selection of superdisintegrants:

Superdisintegrant must follow those conditions in addition to its swelling properties when it is used as an excipient in the tablet formulation. The tablet disintegrant's requirements should be well specified. The perfect disintegrant should possess the following characteristics:

- Poor solubility.
- No tendency to form complexes with the drugs.
- Poor gel formation.
- Good moulding.
- Good hydration capacity.
- Good flow property.
- Good mouthfeel.
- Effective in less quantity.
- Particle size should be small.
- Should be non-toxic.
- It should compatible with other excipients and drug.[15,16,17,18]

2. Superdisintegrants:

To enhance disintegration processes, new materials known as "superdisintegrants" have recently been created.[19,20] Another type of super-absorbing substance with custom-made swelling qualities is superdisintegrants. These materials are designed to swell quickly rather than absorb large volumes of water or aqueous fluids. Superdisintegrants are used to make disintegrable solid dose forms more structurally sound. They are physically scattered throughout the matrix of the dosage form, and when exposed to a moist environment, they expand.

One gram of superdisintegrant absorbs 10-40 g of water or aqueous media on average. Following absorption, swelling pressure and isotropic swelling of the superdisintegrants particles generate stress concentrated zones with a gradient of mechanical characteristics, causing the entire structure to disintegrate, as seen in fig.1.[15]

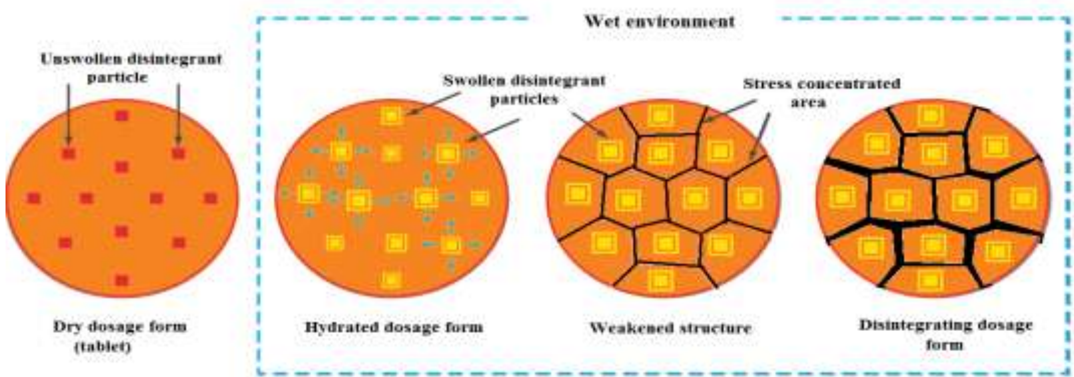

Fig.1: disintegration mechanism of superdisintegrant materials

2.1 Method of Incorporation:

The incorporation of superdisintegrants in the dosage forms are mainly of three types.

Intragranular or during granulation-

The superdisintegrants are mixed with other powders and then granulated in this procedure. Superdisintegrants are thereby absorbed into the granules.

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Advantage-

Easy to add and suitable for direct compression method.[21,22]

Extragranular or before compression-

In this process, the superdisintegrants are mixed with prepared granules before compression.

Advantage-

Suitable for wet granulation process.[21,22]

Incorporation of superdisintegrants at intra- and extra-granulation step:

A portion of the suyperdisintegrants is added to intragranular and a portion to extragranular in this process. In comparison to Type I and Type II, this approach typically yields superior results and more thorough disintegration.[23]

Advantage-

This method is more effective and provides immediate tablet disintegration.[21,22]

2.2 Mechanism of superdisintegrants:[19,24,25,26,27]

The mechanism for breaking the tablets into small pieces and producing a homogeneous suspension is as follows:

1) Swelling
2) Porosity and capillary action(Wicking)
3) Heat of wetting
4) Chemical reaction(Acid-Base reaction)
5) Particle repulsive forces
6) Deformation recovery
7) Enzymatic reaction
8) Combination action(Swelling and wicking)

2.2.1 Swelling

Tablet disintegration is most commonly caused by swelling in both natural and manufactured superdisintegrantas. When the tablet comes into contact with a suitable medium, the first stage in this mechanism is water penetration, followed by swelling of the disintegrant particle, which leads to the generation of swelling force, resulting in tablet disintegration as illustrated in fig.2.

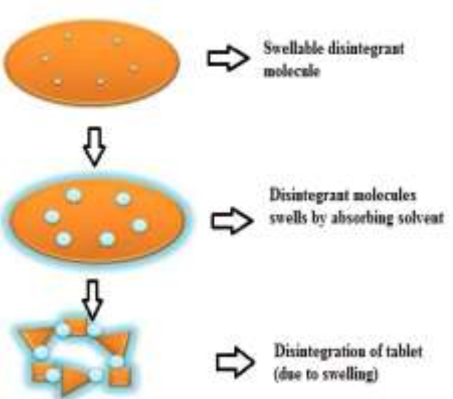

Fig.2: disintegration of tablets by swelling mechanism

2.2.2 Porosity and capillary action(Wicking)

Porosity and capillary action are thought to be responsible for the disintegration action of effective disintegrants that do not swell. Tablet porosity creates routes for liquids to penetrate the tablet. When we immerse the tablet in an appropriate aqueous medium, the medium enters the tablet and replaces the air adsorbed on the particles, weakening the intermolecular link and causing the tablet to disintegrate into tiny particles. The hydrophilicity of the drug/excipient as well as tableting circumstances influence water absorption.Maintenance of a porous structure and low interfacial tension towards aqueous fluid is required for these types of disintegrants, which aids in disintegration by producing a hydrophilic network surrounding the drug particles, as seen in fig.3.

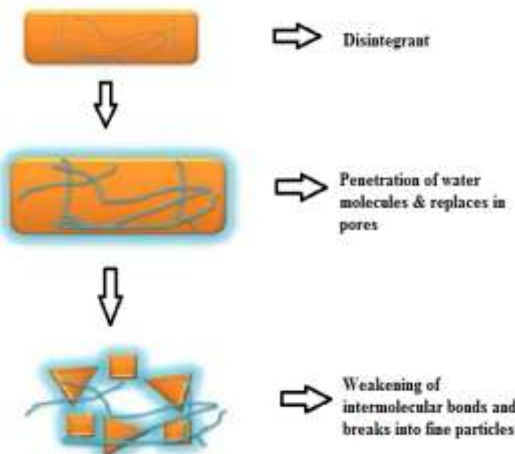

Fig.3: disintegration of tablet by wicking mechanism

2.2.3 Heat of wetting

This method can be used with any disintegrant that has an exothermic feature. When these disintegrants come into touch with appropriate media and get moist, capillary air expansion causes localised stress, resulting in tablet disintegration.[28]

2.2.4 Chemical reaction (Acid-Base reaction)

Due to the interaction of tartaric acid and citric acid with alkali metal carbonates or bicarbonates in the presence of water, the tablet is swiftly broken apart by internal CO2 release in water. The pressure within the tablet causes the tablet to dissolve.

2.2.5 Particle repulsive forces

This approach, which is based on Guyot-particle Hermann's repulsive theory, generates tablet breckdown by using non-swellabledisintegrant particles. Tablet disintegration is caused by electrostatic repulsion between particles, which necessitates the use of water. Researchers discovered that wicking is secondary to repulsion."Tablet in contact with appropriate medium, water enters into the tablet through hydrophilic pores, resulting to the production of a continuous starch-like network that assists in the transfer of water from one particle to another particle and causes hydrostatic pressure," according to Guyot-Hermann repulsion theory. As a result, hydrogen bonds and other forces that hold tablet particles together are broken, as seen in fig.4.[29]

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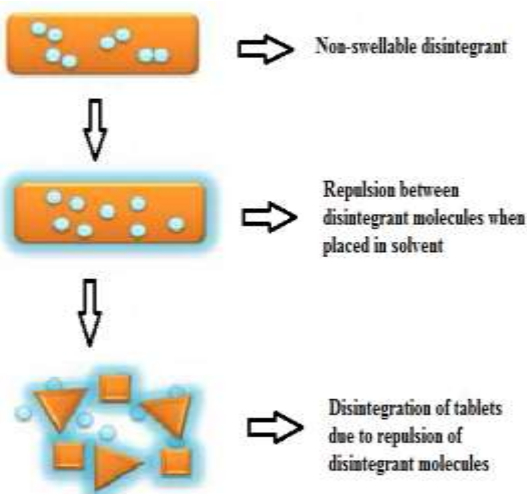

Fig.4: disintegration of tablets by repulsion mechanism

2.2.6 Deformation recovery

Starch grains are supposed to be "elastic" in nature, which means that if they are distorted under pressure, they will revert to their original shape once the pressure is released. However, because to the compression forces used in tableting, these grains are thought to remain permanently damaged and are described as "energy rich," with the energy released when exposed to water. In other words, the potential of “energy rich” starch grains to expand is greater than that of starch grains that have not been distorted under pressure. The activity of most disintegrants is thought to be the result of many mechanisms. Inter-relationships between these fundamental mechanisms are more likely to be the cause.

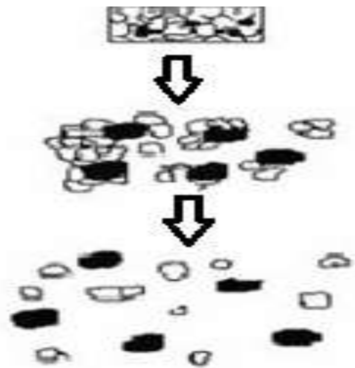

Fig.5: disintegration of tablets by deformation mechanism

2.2.7 Enzymatic reaction

Our bodies include enzymes that function as disintegrators by reducing the binder's capacity to bind. Swelling causes pressure to be applied in the outer direction, causing the tablet to rupture, or fast water absorption creates a massive rise in the volume of granules, promoting disintegration. One body enzymes which help in disintegration of tablets are given in the table 1.

Table 1: examples of enzymes

S. No.	Enzymes
1	Amylase
2	Protease
3	Cellulase
4	Invertase

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2.2.8 Combination action

The swelling and wicking mechanisms of the disintegrant induce the pill to break down.

Example: Crospovidone

2.3 Classification of superdisintegrant

a) Natural superdisintegrant
b) Synthetic superdisintegrant
c) Co-processed superdisintegrant

2.3.1 Natural superdisintegrant

Advantages

- Low cost compared to synthetic and renewable sources.
- Eco-friendly and bio-acceptable.
- Locally available.

2.3.2 Synthetic superdisintegrant

Advantages

- More effective intragranually.
- When compared to starch, it is effective at low concentrations.
- Have a negligible impact on compressibility.
- Have a minor impact on the capacity to flow.

2.3.3 Co-processed superdisintegrant

Excipient granulates are formed by co-processing excipients, which have better qualities than physical mixes of components or individual components. The procedure is used in order to achieve a synergistic change in the particular unwanted trait.

Table 2: name and mechanism of natural superdisintegrants[30,31,32]

S. No.	Name of superdisintegrant	Mechanism
1	Gaur gum	Swelling
2	Xanthum gum	Swelling property
3	Gellan gum	Swelling
4	Loctus bean gum	Swelling and capillary action
5	Agar and treated agar	High strength gelling property
6	Chitin and chitosan	Swelling
7	Mucilage of Lepidus sativum	Swelling
8	Mango peel pectin	Swelling and good solubility
9	Isapghula husk	Swelling
10	Hibiscus rosasinesislinn	Swelling
11	Soy polysaccharide	Swelling
12	Fenugreek seed mucilage	Swelling

Table 3: name and mechanism of superdisintegrants[26,33]

S. No.	Name of superdisintegrant	Mechanism
1	Ion exchange resins	Swelling
2	Chitin and Chitosan	Swelling
3	Crospovidone	Combination of swelling and wicking
4	Croscarmellose Sodium	Swelling and wicking within 10 sec.
5	Calcium silicate	Wicking action
6	CroslinkedAlginic acid	Rapid swelling or wicking
7	Sodium starch	Absorb water quickly
8	MCC and L-HPC	-

Table 4: list of co-processed superdisintegrants

S. No.	Co-processed superdisintegrants
1	Pan Excea MH300G
2	Starlac
3	Ludipress
4	Starcap 1500
5	Ran-Explo-S
6	Ran-Explo-C
7	Ludiflast

3. Conclusion

In the creation of mouth-dissolving tablets, superdisintegrants play a significant role. In an aqueous environment, superdisintegrants aid in the breakage of the tablet into smaller fragments. Superdisintegrants have been examined in terms of selection criteria, benefits, drawbacks, ideal qualities, technique, mechanism, and categorization. The approach of adding superdisintegrants via direct compression has gained appeal among researchers. Mouth dissolving tablet formulations are less complicated than other patented methods due to their simplicity of availability and compactness.

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Evaluation of Protective Role of a Hesperidin on Letrozole induced Polycystic Ovarian Syndrome (PCOS) in Female Rats

Andhalkar Shivani*[1], Chaware Vitthal*[2], Redasani Vivek*[3]

[1]Department of Pharmacology, YSPM's Yashoda Technical Campus Wadhe, Satara, India.
[2]Head of Department (Pharmacology), YSPM's Yashoda Technical Campus, Wadhe, Satara, India.
[3]Principal, YSPM's Yashoda Technical Campus, Wadhe, Satara, India.

Phone no- +91 9970614377; E-mail id :shivaniandhalkar@gmail.com

Abstract : Objective: To evaluate protective effect of Hesperidin (bioflavonoid, found in citrus fruits, such as lemon and orange) on Letrozole induced PCOS in female adult wistar rats. **Methods:** For inducing PCOS, letrozole (1 mg/kg) was administered p.o. for 21 consecutive days, followed by 15-days Hesperidin treatment at the doses 50 mg/kg, 100 mg/kg, and 200 mg/kg, p.o. using 0.5 percent w/v CMC as a vehicle. **Results:** Letrozole caused abnormalities in the ovarian weight, body weight, serum sex steroid profile such as FSH, LH, Testosterone levels and glucose levels. Most of the parameters were restored to normal levels, along with reduction of cysts in the ovaries due to Hesperidin.**Conclusion:** In female wistar rats, Hesperidin had a positive impact on PCOS caused by Letrozole. It had an effect similar to Clomiphene citrate, the most commonly used treatment for induction of ovulation in PCOS.
Keywords : Letrozole, PCOS, Hesperidin, Cysts, Clomiphene citrate.

1. Introduction:

Polycystic ovary (or ovarian) syndrome (PCOS) was first described by Leventhal and Stein in 1935[1]. Polycystic ovary syndrome (PCOS), a set of symptoms affecting women of childbearing age, is assumed to be epidemic in scope. Cysts form in the antral follicles of the ovaries as a result of an imbalance in the proportion of female sex hormones. PCOS is described as when multiple cysts develop in the ovarian follicles as a result of hormonal imbalance. In women, anovulation and the absence of a menstrual cycle inhibit fertilization and reproduction, making pregnancy difficult [2]. PCOS affects 6–10% of all women around the world[3].

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Women with PCOS has several risk factors which are associated with development of uterine cancer including fatness, hyperinsulinemia, diabetes mellitus and abnormal uterine bleeding[4]. The frequency of depression and anxiety is higher in women with PCOS than in general population. Mood disorders are capable of impairing quality of life, which are well-known in young adult women, concerned with fertility, and in women of all ages with respect to obesity, and clinical manifestations of excessive androgen[5].

In rats, various experimental models for PCOS have been developed, including the administration of Estradiol Valerate, DHEA, and an excess of prepubertal androgen[6].Despite the fact that these models cause PCOS, none of them are entirely persuasive and accurately represent the symptoms of human PCOS. Letrozole, a non-steroidal aromatase inhibitor, causes PCOS model that is similar to human PCOS in several respects. It prevents the conversion of testosterone and androstenedione to estradiol and estrone respectively and generates PCOS related syndrome by inducing hormonal imbalance, circulating hyperandrogenism and intra ovarian androgen excess resulting to formation of polycystic ovary[7].Because of the induced elevation of androgen levels in ovaries, follicular atresia and irregular follicular growth are seen[8].Letrozole induction has been linked to hyperglycemia, which can lead to insulin resistance and hyperlipidemia, which also lead to metabolic syndrome[9].Today, a variety of medications are used to treat PCOS and stimulate ovulation. However, extreme side effects such as arthritis, joint or muscle pain, and psychological symptoms have been identified as a result of these therapies. As a result, natural-source medicines with minimal to no side effects are becoming increasingly popular[10].

Hesperidin (5, 7, 30-trihydroxy-40-methoxy-flavanone7-rhamnoglucoside) is a bioflavonoid found in citrus fruits like orange and lemon, as well as plant-derived beverages like tea and olive oil, which have traditionally been used in herbal medicine[11]. Hesperidin has wide range of pharmacological properties, such as antioxidant[12], anti-inflammatory, antihyperlipidemic[13], properties.

Hesperidin modulates the different hallmarks of cancer notably cell death, inflammation and oxidative stress mechanism.Hesperidin is also one of the most essential bioflavonoid present in the Citrus genus (Rutaceae)[14].In DMI rats, hesperidin has a considerable reduction in total blood lipid profiles and plasma insulin concentrations, as well as anti-hyperglycemic and hypolipidemic activity[15]. Due to the stated activities, we hypothesised that Hesperidin could be useful in the treatment of Letrozole-induced PCOS in this investigation.

2. Materials and Methods:

2.1. Experimental animals

Virgin, cyclic, adult female Wistar Albino rats (150–200 g) were employed for the study. These animals were procured from registered breeder and acquainted in the quarantine area for one week.After acquaintation, animals were transferred to the standard laboratory conditions and allowed to acclimatise for two weeks in animal house of YSPM’s Yashoda Technical Campus, Pharmacy, Wadhe, Satara. During the experimental study allanimals were caged in standardpolypropylene cages with maintained controlled environmentof 22 ±3℃ temperature, 50 ± 15% humidity and a 12 h light/dark cycle. They were fed with standard pellet diet and water provided*ad libitum*. The study was duly approved by Institutions AnimalEthics Committee(IAEC) for the use of animals and care of the animalswas carried out as per the guidelines of committee for the purposeof control and supervision of experiments on animals (CPCSEA).

2.2. Drugs and Reagents

Hesperidin was acquired from OZONE® INTERNATIONAL (INDIA). Letrozole was obtained from Sun Pharmaceutical Ind Limited.Clomiphene Citrate (Fertomid-50) tablets were procuredfrom Cipla, India. All other chemicals used were of analytical grade. Theserum follicle stimulating hormone (FSH), luteinizing hormone (LH) and Testosterone were measured via Enzyme Linked Immunosorbent Assay(ELISA) with the help of commercial kits (ELISA kit).Blood glucose level was measured using Accu-Check Active glucometer.

2.3. PCOS induction

All the experimental animals except control group were orally administered with Letrozole at a dose of 1 mg/kg dissolved in 0.5% Carboxy Methyl Cellulose (CMC) once daily for 21 days[7]. Control group received

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vehicle only (0.5% CMC). Vaginal Smears were collected daily and evaluated microscopically using 0.1% Crystal violet stain (prepared by diluting 0.1 g of crystal violet in 100 ml of double distilled water followed by filtration through whatman filter paper) and examined under X10 objective lens of laboratory microscope to confirm the induction of PCOS.

2.4. Study design

Thirty-six female Albino Wistar rats were divided into six groups: group 1 (control group), group 2 (PCOS induced group), group 3 (standard group), groups 4, 5 and 6 (treatment groups). Following Letrozole administration, standard group was administered with Clomiphene Citrate at a dose of 1 mg/kgp.o.in 0.5% CMC and treatment groups 4, 5 and 6 were administered Hesperidinat the dose of 50 mg/kg, 100 mg/kg and 200 mg/kg p.o.respectively in 0.5% CMC for 15 days i.e., from day 22nd to 36th day. At the end of the treatment animals were fasted overnight and anaesthetized with diethyl ether. Blood was collected by puncturing retro-orbital sinusthen by centrifugation method serum was separated and used for estimation of hormones.

2.5. Biochemical estimations

2.5.1.Hormonal assay

The serum luteinizing hormone (LH), follicle stimulating hormone (FSH), and Testosterone were measured via Enzyme Linked Immunosorbent Assay(ELISA) with the help of commercial kits (ELISA kit).

2.6. Ovarian histomorphology

The excised ovaries were fixed in 10% Formalin. According to histological procedure, they were subjected to tissue processing by washing with water which was followed by dehydration through an ascending ethanol series, then cleared through xylene. Then paraffin embedding method was used. The blocks were sectioned at 5 μm thickness using microtome and were mounted on slides coated with poly-lysine. These blocks were stained with hematoxylin-eosin(HE), dehydrated, cleared and mounted on DPX mountantunder glass cover slips. The light microscope (100X) was used for observation of slides which was connected to a camera to capture images[16].

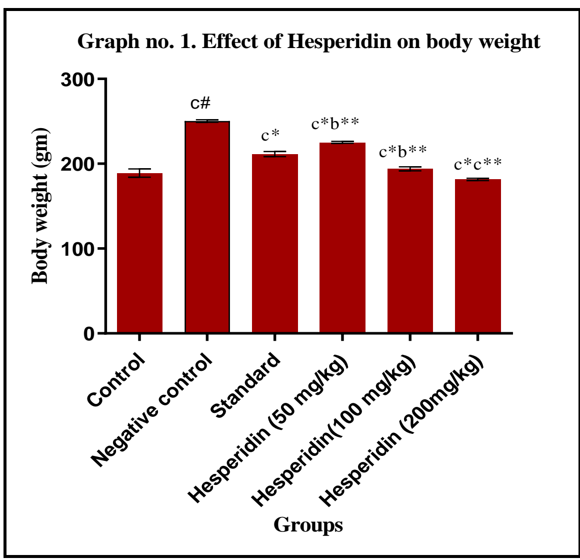
2.7. Statistical analysis

The statistical analysis was carried out with Graph pad prism 5.0 software. The data was statistically analyzed using one-way ANOVA method followed by Tukey's multiple comparison tests and p values ($p<0.05$, $p<0.01$, $p<0.001$)were considered to be statistically significant.

3. Results

3.1. Body weights

When body weight compared with control group, negative controlgroup showed significant elevation ($p<0.001$) in body weight. When standard and all treatment groups of hesperidin (50mg/ kg, 100mg/kg, 200mg/kg) compared to the negative group, the body weight decreased considerably ($p<0.001$).Low and Intermediate dose of hesperidin significantly ($p<0.01$) reduced the body weight as compared to standard group. High dose of hesperidin showed significantly ($p<0.001$) reduction in body weight as compared to standard group. (Graph no. 1)

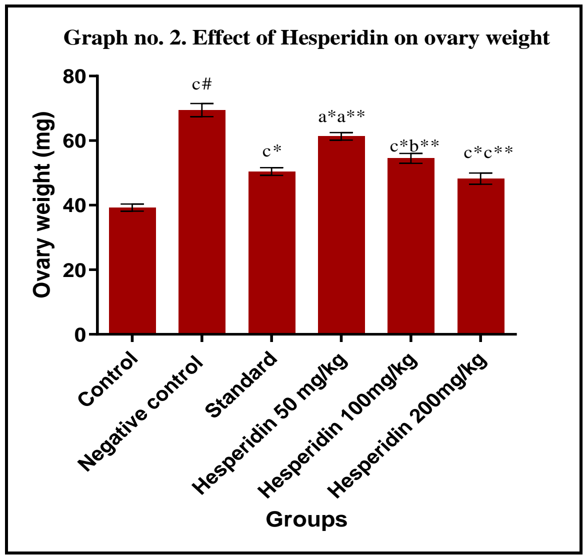


#= Data compared with control group
***= Data compared with negative control group**
****=Data compared with standard group**
a=p<0.05, b=p<0.01, c=p<0.001

3.2. Organ weights

3.2.1. Ovary weights

In terms of ovarian weights, there was significant increase in ovarian weight in negative group as compared with control group. After treatment with standard drug there was significant ($p<0.001$) decrease in ovarian weight as compared to negative group. When negative control groupcompared with intermediate and high dose of hesperidin, then it shows significantly ($p<0.001$)reduced ovarian weight. When low dose of hesperidin compared with standard and negative control group, then it indicated less significant decrease in ovary weight; whereas intermediate and high dose showed significantly ($p<0.01$ and $p<0.001$) decrease in weight of ovary respectively.(Graph no. 2)



#= Data compared with control group
***= Data compared with negative control group**
****=Data compared with standard group**
a=p<0.05, b=p<0.01, c=p<0.001

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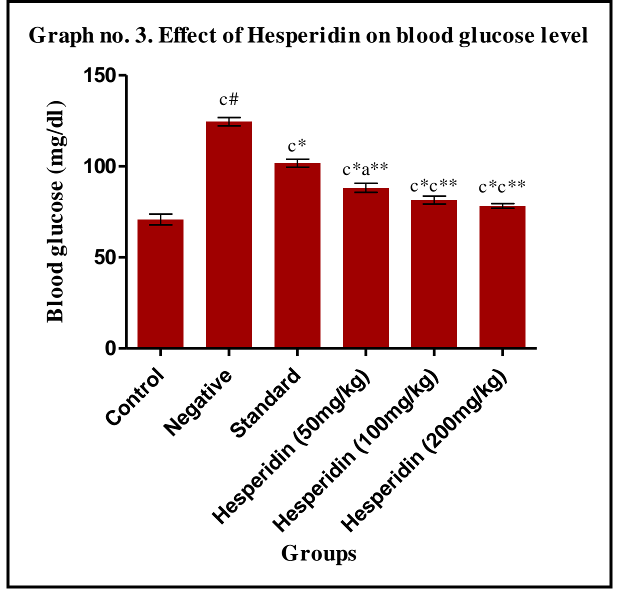
Table no. 1. Evaluation of Hesperidin on body weight and ovary weight of Letrozole induced PCOS in female rats

Group no.	Groups	Body weight(gm)	Ovary weight (mg)
1	Control	189.0±4.92	39.26±1.10
2	Negative	250.5±1.54[c#]	69.47±2.00[c#]
3	Standard	211.3±3.04[c*]	50.41±1.20[c*]
4	Hesperidin(50 mg/kg)	225.2±1.11[c*b**]	61.33±1.15[a*a**]
5	Hesperidin(100 mg/kg)	194±2.40[c*b**]	52.49±1.53[c*b**]
6	Hesperidin(200 mg/kg)	181.5±1.34[c*c**]	48.22±1.74[c*c**]

Control: CMC; Negative control: Letrozole; Standard: Clomiphene citrate; Low dose: Hesperidin 50mg/kg; Intermediate dose: Hesperidin 100mg/kg; High dose: Hesperidin 200 mg/kg

3.3. Fasting Blood Glucose(FBG) levels

Negative group exhibited significantly ($p<0.001$) elevated blood glucose level as compared to control group. When standard and all doses of hesperidin compared with negative group it showed significant ($p<0.001$) reduction of blood glucose level. Intermediate and high dose of hesperidin exhibited significantly($p<0.001$) decreasing level of blood sugar as compared with standard group. Low dose of hesperidin compared with standard then it indicated less significant decrease in ovary weight. (Graph no. 3)



#= Data compared with control group
***= Data compared with negative control group**
****=Data compared with standard group**
a=p<0.05, b=p<0.01, c=p<0.001

Table no. 2. Evaluation of Hesperidin on Fasting blood glucose(FBG) of Letrozole induced PCOS in female rats

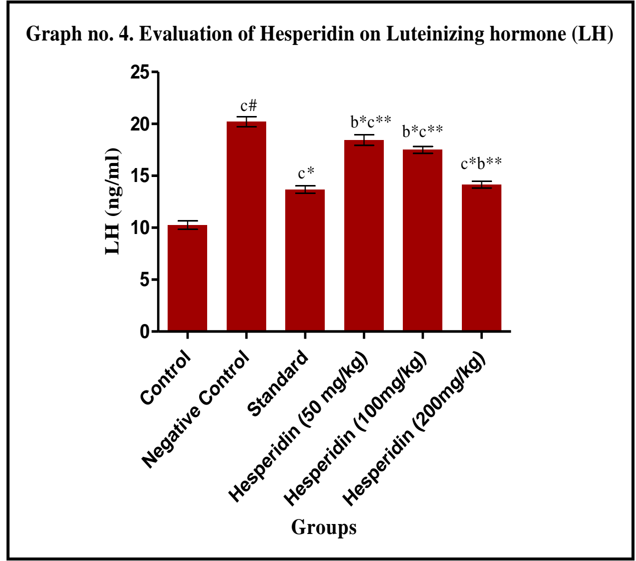
Group no.	Groups	Blood glucose (mg/dl)
1	Control	70.83±2.97
2	Negative	124.5±2.26$^{c\#}$
3	Standard	101.7±2.19^{c*}
4	Hesperidin(50 mg/kg)	88.17±2.46^{c*a**}
5	Hesperidin(100 mg/kg)	81.50±2.20^{c*c**}
6	Hesperidin(200 mg/kg)	76.33±1.27^{c*c**}

Control: CMC; Negative control: Letrozole; Standard: Clomiphene citrate; Low dose: Hesperidin 50mg/kg; Intermediate dose: Hesperidin 100mg/kg; High dose: Hesperidin 200 mg/kg.

3.4. Serum sex steroid profile

3.4.1.Evaluation of Hesperidin on Luteinizing hormone(LH) in Letrozole induced PCOS female rats

The serum levels of LH were remarkably increased in negative group ($p<0.001$) as compared with control group. All doses of Hesperidin showed significant reduction in LH level when compared with negative control group. Hesperidin (50mg/kg and 100mg/kg) showed significantly ($p<0.01$) decreased LH level as compared with standard. High dose of hesperidin shows decreased LH level significantly($p<0.01$) when compared with standard group. (Graph no.4)



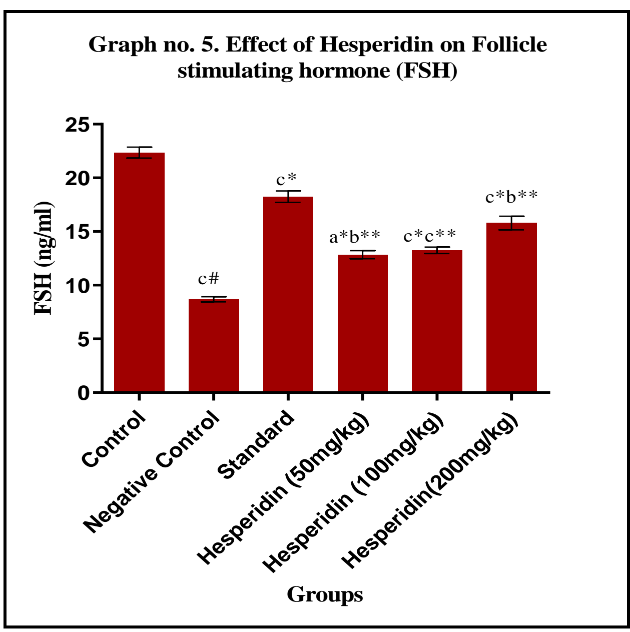
Graph no. 4. Evaluation of Hesperidin on Luteinizing hormone (LH)

#= Data compared with control group
***= Data compared with negative control group**
****=Data compared with standard group**
a=p<0.05, b=p<0.01, c=p<0.001

3.4.2. Evaluation of Hesperidin on Follicle stimulating hormone(FSH) in Letrozole induced PCOS female rats

The serum levels of FSH were significantly reduced in negative group of animals when compared with control group of animals.When both intermediate and high dose of hesperidin (100mg/kg and 200mg/kg) was compared with negative group it showed significant ($p<0.001$) elevation in FSH levels. Intermediate and high dose of hesperidin exhibited significant ($p<0.001$) increased FSH levels in comparison with standard group.

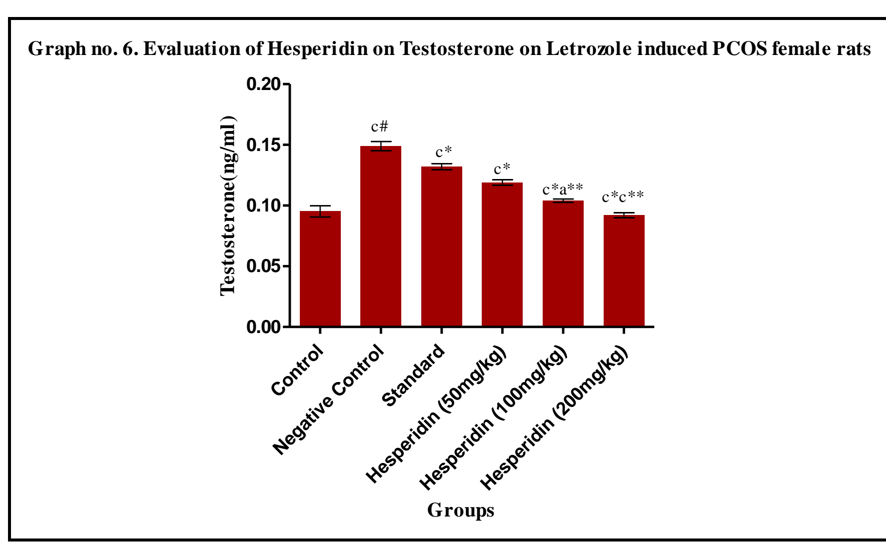
Low dose of hesperidin compared with standard and negative control then it indicated less significant decrease in FSH level. (Graph no.5)



#= Data compared with control group
***= Data compared with negative control group**
****=Data compared with standard group**
a=p<0.05, b=p<0.01, c=p<0.001

3.4.3. Evaluation of Hesperidin on Testosterone in Letrozole induced PCOS female rats

Testosterone significantly (p<0.001) raised in Letrozole induced group as compared with control group whereas standard and low, intermediate and high dose group of hesperidin significantly (p<0.001) reduced the levels of testosterone. Intermediate dose and high dose group of hesperidin significantly (p<0.05 and p<0.001 respectively) lowered the level of testosterone when compared with standard.(Graph no. 6)



#= Data compared with control group
***= Data compared with negative control group**
****=Data compared with standard group**
a=p<0.05, b=p<0.01, c=p<0.001

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Table no. 3. Evaluation of Hesperidin on Luteinizing hormone(LH), Follicle stimulating hormone(FSH) and Testosterone on Letrozole induced PCOS female rats

Group no.	Groups	Luteinizing hormone(LH) (ng/ml)	Follicle Stimulating Hormone (FSH) (ng/ml)	Testosterone (ng/ml)
1	Control	10.25±0.40	22.36±0.51	0.095±0.004
2	Negative	20.21±0.48$^{c\#}$	8.69±0.23$^{c\#}$	0.149±0.003$^{c\#}$
3	Standard	13.68±0.35^{c*}	18.25±0.36^{c*}	0.132±0.002^{c*}
4	Hesperidin(50 mg/kg)	18.44±0.51^{b*c**}	12.85±0.37^{a*b**}	0.119±0.002^{c*}
5	Hesperidin(100 mg/kg)	17.39±0.34^{b*c**}	13.25±0.29^{c*c**}	0.104±0.001^{c*a**}
6	Hesperidin(200 mg/kg)	14.14±0.32^{c*b**}	15.80±0.63^{c*b**}	0.092±0.002^{c*c**}

Control: CMC; Negative control: Letrozole; Standard: Clomiphene citrate; Low dose: Hesperidin 50mg/kg; Intermediate dose: Hesperidin 100mg/kg; High dose: Hesperidin 200 mg/kg.

3.5. Histopathological changes

3.5.1. Histopathological observation of ovaries in Control group

Section of ovaries from control group animals showed healthy follicles with oocyte at different stages of development. The primary, secondary and tertiary follicles indicated by arrows. (Figure 1)The photograph shows normal developing stages of follicles in ovary.

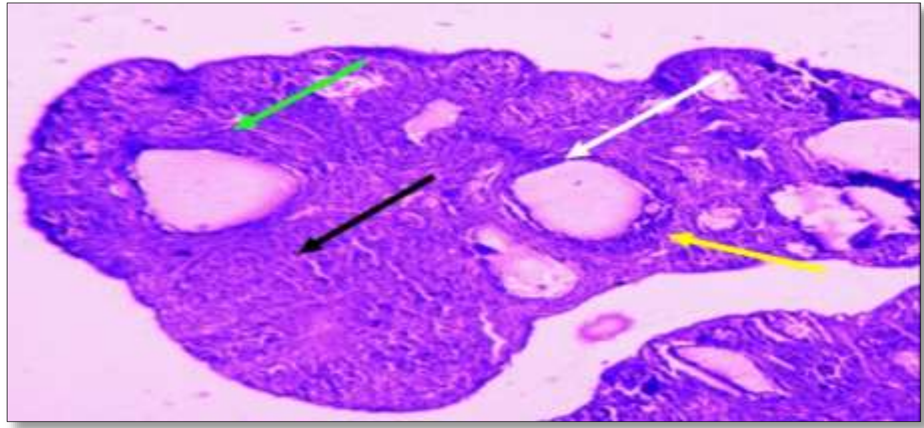

Fig no. 1 Histopathological observation of ovaries in Control group

Normal: Primary (white arrow), secondary (yellow arrow), tertiary (green arrow), corpus luteum (black arrow) (H&E stain100X)

3.5.2. Histopathological observation of ovaries in Negative Control group

Letrozole treated rats exhibited numerous cysts, with a very thin or no granulosa layer (Figure 2).Corpora lutea were completely absent indicating anovulation. Few follicles were observed at their early stages of development. In addition, they were accompanied withatretic follicles containing fluid filled antrum.

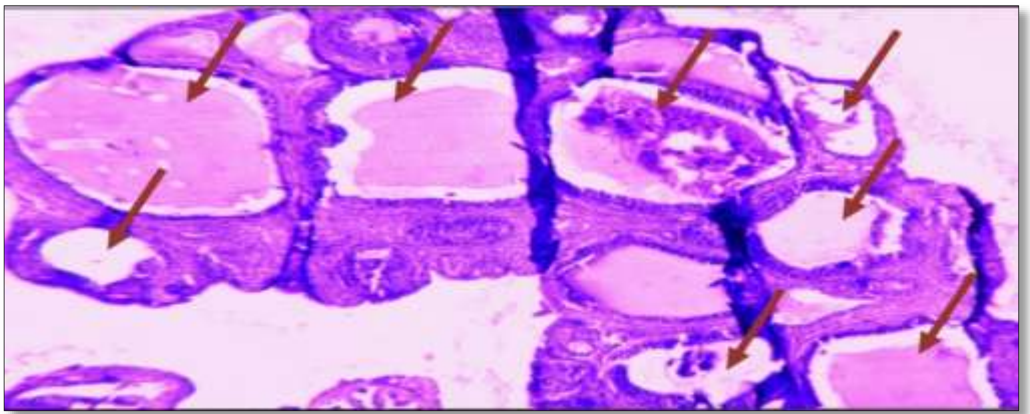

Fig no. 2. Histopathological observation of ovaries in Letrozole induced (Negative control) group

Negative: Cystic follicles (Brown arrow)

3.5.3.Histopathological observation of ovaries in Standard group

Clomiphene citrate treatment led to disappearance of cysts and appearance of healthy follicles and corporalutea. Decrease in cyst as compared to negative control group and shows developing follicles. (Figure 3)

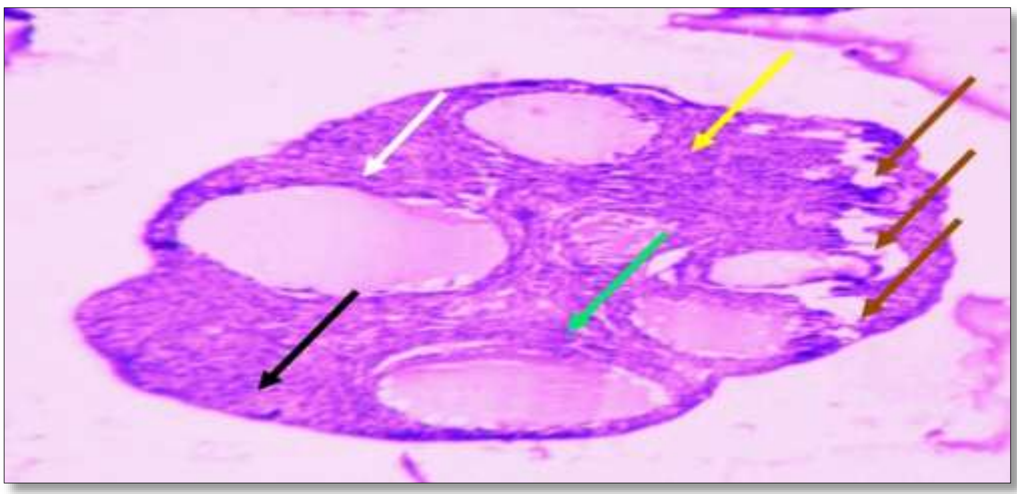

Fig no. 3. Histopathological observation of ovaries in Standard group

Standard: Primary (white arrow), secondary (yellow arrow), tertiary (green arrow), corpus luteum (black arrow), cystic follicles (brown arrow)

3.5.4. Histopathological observation of ovaries in Low dose (50 mg/kg) of hesperidin

Sections from low dose of hesperidin (50 mg/kg) group exhibited follicles larger in size and few corpora lutea. It also shows developing stages of follicles. (Figure 4)

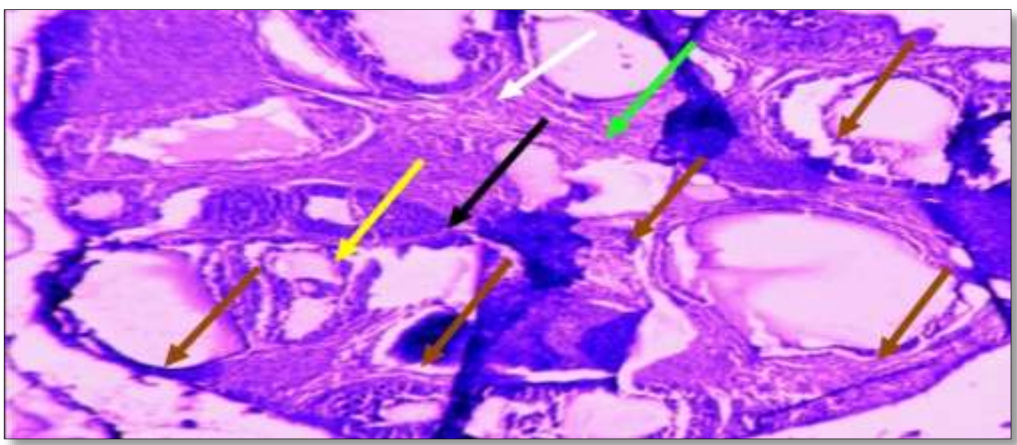

Fig no. 4. Histopathological observation of ovaries in Low dose (50 mg/kg) of hesperidin

Low Dose: Primary (white arrow), secondary (yellow arrow), tertiary (green arrow), corpus luteum (black arrow), cystic follicles (brown arrow)

3.5.5. Histopathological observation of ovaries in Intermediate dose (100 mg/kg) of hesperidin

Sections from intermediate dose of hesperidin (100 mg/kg) group exhibited few cysts and few corpora lutea. It also shows well differentiated developing stages of follicles. (Figure 5)

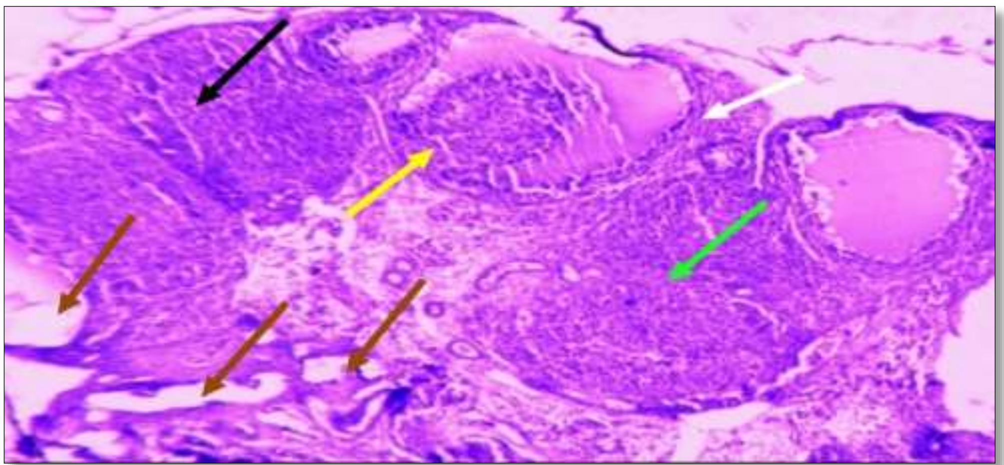

Fig no. 5. Histopathological observation of ovaries in Intermediate dose (100 mg/kg) of hesperidin

Intermediate Dose: Primary (white arrow), secondary (yellow arrow), tertiary (green arrow), corpus luteum (black arrow), cystic follicles (brown arrow)

3.5.6. Histopathological observation of ovaries in High dose (200 mg/kg) of hesperidin

Cysts were very less in number and normal sized healthy follicles at differentdevelopmental stages with oocytes were found in section fromhigh dose (200 mg/kg) group (Figure 6). Also with the high dosemany corpora lutea and antral follicles with clearly differentiated oocyte, granulosa cell layer were observed.

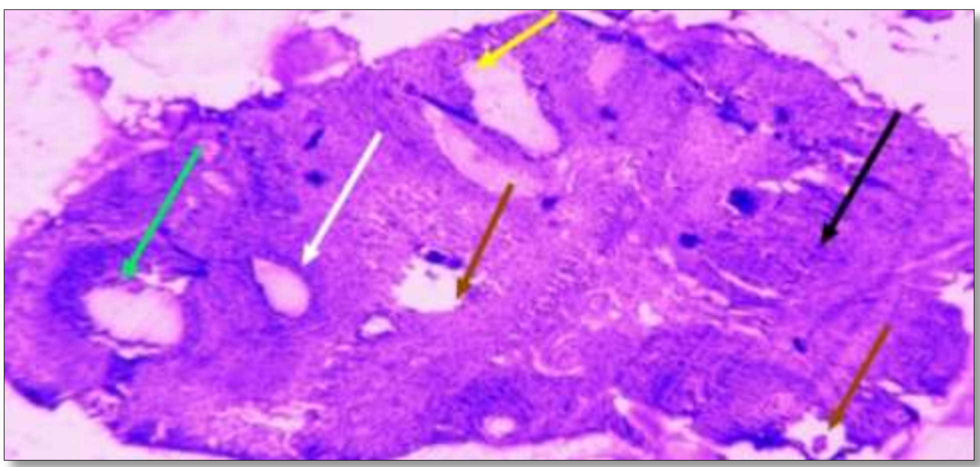

Fig no. 6. Histopathological observation of ovaries in High dose (200 mg/kg) of hesperidin

High Dose: Primary (white arrow), secondary (yellow arrow), tertiary (green arrow), corpus luteum (black arrow), cystic follicles (brown arrow)

4. Discussion

In this research, using the inhibitor of non-steroidal aromatase i.e. Letrozole, PCOS condition was developed in animals which presented biochemical and histological changes. The clinical and biochemical characteristics caused by PCOS in rats were investigated in this research.

The working of Letrozole induced PCOS model was confirmed by regular examination of vaginal smears and persistent vaginal cornification[17]. PCOS-induced rats enhanced body weight owing to abdominal fat deposition. But the body weight was considerably reduced by the therapy with hesperidin (50mg/kg, 100mg/kg, 200mg/kg). The weight of ovariesin negative group was greater than rats incontrol group, according to previous studies. The hesperidin therapy(50mg/kg,100mg/kg, and 200mg/kg)prohibited further rise in ovarian weight. The weight of ovaries in the negative group was greater than that of control rats according toobservations. Hesperidin therapy(50mg/kg,100mg/kg, and 200mg/kg) stopped further ovarian weight gain.

Letrozole induction of PCOS led in testosterone and LH levels being elevated while FSH level decreased. This imbalance in hormonal level led to an inconsistent cycle of estrous. The same circumstance have been noted in this research. Letrozole induced rats showed considerably higher concentrations of testosterone and LH when compared to control. Standard drug clomiphene citrate and hesperidin (100mg/kg, 200mg/kg) treated animals showed substantially reduced testosterone and LH levels. Hesperidin treatment showed FSH level elevation[18-20].

PCOS is also strongly linked with type-2 diabetes mellitus and insulin-resistant hyperglycemia[22]. In our analysis, there was a marked rise in blood glucose level in the negative group relative to the control group. Oral administration of hesperidin considerably inhibited increased blood sugar levels, indicating the impact of hesperidin on insulin resistance and diabetic conditions. Hesperidin showed beneficial effect against hyperglycemia as well as hyperlipidemia.

In ovaries, enhanced oxidant concentrations may change the steroidogenesis resulting in enhanced rates of androgen production and polycystic ovaries.[20-21] Many studies reported that oxidative stress is one of the various pathologicalfactors in women with PCOS[23].The histopathological report of Letrozole induced rats revealed the existence of polycysts in the ovary. If negative group compared to control group then negative group showed that more than two cysts were formed in the ovary. After therapy with hesperidin the cystic follicles reduced and also shows healthy follicles at different stages of development. This implies a marked recovery of ovarian tissue by hesperidin.

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5. Conclusion

In normalizing the various parameters of PCOS condition in rats, the impact of hesperidin therapy with medium (100mg/kg) and high (200mg/kg) dose was observed to be similar with standard treatment.

In letrozole induced polycystic ovarian animals, hesperidin recovered the serum hormonal levels such as FSH,LH, and Testosterone, glycemic condition, along with body weight and ovarian weight. Thus, hesperidin might be helpful in managing PCOS condition due to restoration of irregular follicular phase and abnormal changes in the ovaries of rats.

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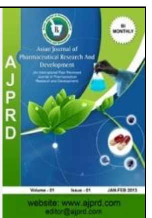

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Research Article

Evaluation of Hepatoprotective Activity of Leaves Extract of *Pithecellobium Dulce* In Experimental Animals

Komal Sul*[1], Vitthal Chaware [2], Vivek Redasani [3]

[1]Department of Pharmacology, YSPM's Yashoda Technical Campus Wadhe, Satara, India.

[2]Head of Department (Pharmacology), YSPM's Yashoda Technical campus, Wadhe , Satara

[3]Principal, YSPM'sYashoda Technical Campus, Wadhe , Satara, India

ABSTRACT

Objective: To evaluate the hepatoprotective activity of leaves extract of *Pithecellobiumdulce* in experimental rats.
Methods: For the generation of Hepatotoxicity ,paracetamol (2g/kg) was administered orally for 7days, accompanied by a 7-day dose of Ethanolic extract of *Pithecellobiumdulce*leaves(100mg/kg, 200mg/kg, and400mg/kg, p.o.)using 1 % w/v CMC as a vehicle.
Results: From the present investigation, it was observed that ethanolic extract of *Pithecellobium dulce* have shown significant reduction in serum glutamate oxaloacetate transminase (SGOT),serum glutamate pyruvate transminase (SGPT),alkaline phosphatase (ALP) triglyceride, bilirubin level in paracetamol induced hepatotoxicity.
Conclusion: The findings in this study revealed the effectiveness of ethanolic extract of *Pithecellobiun dulce* against hepatotoxicity activity.

Keywords: *Pithecellobium dulce* ,Hepatotoxicity, Silymarin, Paracetamol, SGOT,SGPT,ALP

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***Address for Correspondence:**

Komal Sul, Department of Pharmacology, YSPM's Yashoda Technical Campus Wadhe, Satara, India.

1. INTRODUCTION:

Hepatotoxicity is also termed as aquired high blood triglycerides; high bilirubin,highserum glutamate pyruvate transminase (SGPT); high serum glutamate oxaloacetate transminase (SGOT). It is an elevation of one or more of the plasma lipids, including triglycerides, cholesterol,always corresponding with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSH levels[1]. In addition serum levels ofmany biochemical markers like bilirubin,triglyceride, alkaline phosphatase(ALP),Serum glutamate pyruvate transminase(SGPT),Serum glutamate oxaloacetate transminase(SGOT), triglycerides, cholesterol are exalted.The liver is the principal glandular organ in the body and has more functions than any other human organ.Liver is not only the second-largest organ of the body but also the largest gland. Liver is weighing about 1.4 kg in grown person and is underline to the diaphragm occupying most of the right hypochondriac and a part of the epigastric region of abdominopelvic cavity[2].Liver deals with many pathways which include energy provision ,nutrient supply,relevant to growth, fight against disease, and reproduction are corresponded with liver[3,4].The liver has an inventory task of maintaining the body's metabolic homeostasis. The liver is expected to perform physiological functions as well as protects against the hazards of chemicals and harmful drugs[5].Though tremendous scientific advancement in the field of hepatology , liver problems are on the rise. Jaundice and hepatitis are two major hepatic disorders and having higher mortality rate[6].In the makett , for the treatment of liver

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disorders there is only few hepatoprotective drugs are available .

Pithocellobium dulce Benth belongs to the family of Leguminasae.It is a small to medium sized ,evergrren and found in the India and Southern Asia.Locally *Pithocellobium dulce* is known by various names at different region such as Vilayti Babul in hindi and Vilayti chinch in Marathi and in English it is known as Manila tamarind[7]. The fruit of plant *Pithecellobium dulce* is well known as edible fruits and they have been consumed for various ailments in a traditional manner.The Fruits are greenish-brown to red-pinkish, indehiscent pods and linear, curved legumes (pods) that range in length from 10 to 13 cm. usually,pods are 10-15 cm; the colour becomes reddish brown.pods are irregular in shape and flattened,Each pods contain 5-10 shiny seeds, set in spirals of 1 to 3 whorls and strangled between the seeds (lomentaceous). The seeds are black in colour and shiny nature and 1cm long surrounded by an edible white pulp. The pod is peeled on both sides[8] (Orwa et al., 2009).*Pithecellobium dulce leaves* has been reported to contain Quercetin, Kaempferol, cyclitol, dulcitol afezilin[9]. *Pithecellobium dulce* also contains Quercetin 3-O-rhamnoside ,Seven saponins named Pithedulosides A-G,Pinitol,triterpenoids,glycoside etc. Literature survey indicates that *Pithecellobium dulce* possesses Antidiarrhoeal activity[10],Anticonvulsant activity[11], Adulticidal activity[12], Hypolipidemic activity[13], Anti-oxidant activity[14], Anti-diabetic[15], Antifungal Activity[16], Analgesic[17] ,Anti-inflammatory[17] ,Larvicidal and ovicidal activities[18] and antimicrobial activity[19]etc .Therefore, the present investigation was under taken to evaluate hepatoprotective effect of ethanolic extract of *Pithecellobium dulce* in paracetamol induced hepatotoxicty in rats which has not been earlier reported . It is our belief that this examination will take us another step forward in our quest to understand the mechanism of action of *Pithecellobium dulce* in prevention and medicaments of liver related diseases.

2. MATERIALS AND METHODS:

2.1.Drugs& chemical

Paracetamol was acquired from S.D.fine.chemialsMumbai.Silymarin(silybon140,Cipla Ltd.) was purchased in a tablet form at strength 140 mg. All other chemicals and reagents used were of analytical grade and aquired from approved chemical suppliers. The serum glutamate oxaloacetate (SGOT), Serum glutamate pyruvate transminase (SGPT) and Serum alkaline phosphate (ALP)and Total bilirubin were measured with the help of commercial kits.

2.2. Collection and extraction of plant material

The fresh leaves of *Pithecellobium dulce* was collected form local area of, Solapur District, Maharastra, India and were identified by a botanist. They were washed with tap water and dried leaves were powdered and passed through sieve for coarse powder. This shaded dried powdered was extracted successively with (1:6)Petrolium ether, Chloroform, Ethanol in a soxhlet apparatus allowed to stand for a period of 18 hr, and filtered by using Whatman filter paper (No. 1). The filtrate was concentrated at 45°C in a water bath for complete dryness. Crude extract obtained was stored at 4°C for further use.

2.3. Experimental animals

The complete experiment was carried out using 36 wistar albino rats of both sex weighing 150 - 200g.The study protocol was approved by Institutional Animal Ethics Committee (IAEC) ,YTC, YSPM, Satara. The animals were aquired from registered breeder and familiarized in the quarantine area for one week. Animals were housed in clean polypropylene cages in a controlled room temperature 22°C ± 2°C, relative humidity of 50 ± 15% and 12 hr dark/ 12 hr light cycle at our Institution's animal house and allowed to aculturate for two weeks. The animals were fed with water and standard pellet diet ad libitum. Animals were maintained as per Committee for the Purpose of Control and Supervision of Experiments on Animals Guidelines.

2.4. Preparation of standard drug

Silymarin tablets were crushed into powder, dissolved in distilled water at dose 200mg/kg b. w. and administered orally.

2.5. Induction of hepatotoxicity

All the experimental animals excepts control group ,were administered Oraly with Paracetamol at a dose of 2 gm/kg dissolved in 1% Carboxy Methyl Cellulose(CMC).

2.6. Experimental design

A total 36 wistar albino rats of either sex were randomly divided into 6 groups containing 6 animals in each group. Group I (Normal control) did not receive any treatment apart from vehicle 10ml/kg b. w. /day for 7 days. Group II (Negative control) were induced with 2.0g/kg b. w. dose of Paracetamol without treatment . Group III(Standard control) were induced with 2.0g/kg dose of Paracetamol and treated with silymarin at a dose of 200mg/kg b. w. /day for 7 days. Group IV, V and VIwere induced with 2.0g/kg dose of Paracetamol and treated with test drug at dose 100 (low), 200 (medium) and 400 (high) mg/kg b. w. /day for 7days respectively.

2.7.Blood Sample Collection

At end of the experimental period animals were kept fasted over night and anaesthetized with diethyl ether. Blood samples were collected serially by retro orbital puncture.The blood was allowed to clot for 30 min at room temperature then serum was separated by centrifugation and used for biochemical parameters like serum glutamate

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oxaloacetate (SGOT),Serum glutamate pyruvate transminase (SGPT) and Serum alkaline phosphate (ALP),Bilirubin, Triglycerides.

2.8.Evaluation parameters

2.8.1. Physical Parameter

Determination of wet liver weight:

Animals were sacrificed and livers were isolated and washed with saline and weights determined by using an electronic balance.The liver weight were expressed with respecte to its body weight i.e.gm/100gm.

Determination of Wet liver Volume:

After recording the weight all the livers were dropped individual in a measuring cylinder containing a fixed volume of distilled water or saline and the volume displaced was recorded.

2.8.2. Biochemical parameters

The principle, details of kits and methodology used in the estimation of various bio-chemical parameters by Autoanalyser in the present investigation are as follows

- Estimation of AST
- Estimation of ALT
- Estimation of ALP
- Estimation of bilirubin
- Estimation of triglycerides

2.8.3. Liver histopathology

The fixed specimens of liver were evolved by washing through running tap water, dehydration through ascending grades of alcohol, clearing through xylene and embedding completely with in paraffin into blocks. The serial sections of not exceeding 3 mm thickness were cut using microtome and were mounted on polylysine coated slides, removal of paraffin from tissues by using xylene, rehydrated and stained with hematoxylin and eosin, dehydrated, cleared and mounted on DPX under glass cover slips. The slides were then observed under light microscope which was connected to a camera to capture images.

2.9.Statistical analysis

The statistical analysis was carried out with Graphpad prism 5.0 software. The data was statistically analyzed using one-way ANOVA followed by Tukey's multiple comparison tests and $p < 0.05$ was considered to be statistically significant.

3. RESULTS AND DISCUSSION

3.1.Yield of the extract

The yield of the extract was found to be 24.35%. Further preliminary phytochemical screening revealed the presence of flavonoids, saponins, phenol, carbohydrates, tannins, alkaloids, sterols and glycoside.

3.2.Liver weight and Liver volume

Paracetamol treatment in rats showed the hepatic damage which was evident by increase in the liver weight and volume.when treated with standard (Silymarin) showed good reduction in weight and volume of liver.

Table1: Effect of ethanolic extract of Pithecellobiumdulce on Liver weight and volume of Paracetamol induced hepatotoxicity in experimental animals.

Sr. No	Experimental group	Liver weight gm/100gm	Liver volume ml/100gm
1	Normal control	2.82 ±0.11	5.62 ±0.24
2	Negative control	4.81 ± 0.12	9.62 ±0.17
3	Standard control	2.87 ±0.09	6.43 ±0.20
4	Test I	3.73 ±0.19	8.78 ±0.16
5	Test II	3.30 ±0.13	7.51 ±0.17
6	Test III	3.12 ±0.10	6.93 ±0.13

Normal control: distilled water ; Negative control : Paracetamol; Standard control: Silymarin; Test I :EEPD(100mg/kg) ; Test II EEPD (200mg/kg)Test III EEPD(400mg/kg)

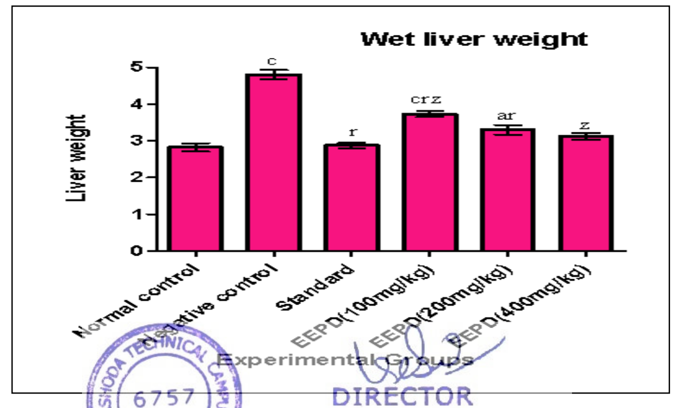

Figure: 1.Effect of ethanolic extract of Pithecellobiumdulce leaves on Wet liver weight

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Values represented mean ± SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. a<0.05, b<0.01, c<0.001; Data compared with normal control; p<0.05, q<0.01, r<0.001 Data compared with negative control; x<0.05, y<0.01, z<0.001 Data compared with standard control (by one way ANOVA followed by Tukey's multiple comparison test).

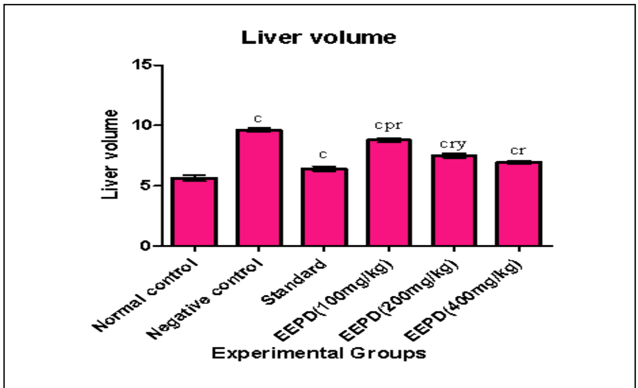

Figure 2: Effect of ethanolic extract of Pithecellobiumdulce leaves on Wet liver volume

Values represented mean ± SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. a<0.05, b<0.01, c<0.001; Data compared with normal control; p<0.05, q<0.01, r<0.001 Data compared with negative control; x<0.05, y<0.01, z<0.001 Data compared with standard control (by one way ANOVA followed by Tukey's multiple comparison test).

3.2. Liver functional test

Paracetamol administration developed acute hepatotoxicty in rats by significantly increasing the level of triglycerides, SGOT,SGPT,ALP,Bilirubin level in rats treated with paracetamol group as compared to normal control group.

Effect of EEPD on AST,ALT,ALP levels in paracetamol induced hepatotoxicity in wistar rats:

Rats treated with paracetamol developed a significant liver damage observed as elevated serum levels of hepatospecific enzymes like aspartate amino transferase ,Alaninetransferase,when compared with normal control.Medicaments with silymarin showed protection against paracetamol induced hepatotoxicity.Test groups treated with ethanolic extract of leaves of pithecellobiumdulce showed significant effect which can be comparable with toxic control. In One way ANOVA Dunnet's test indicates a significant reduction on elevated serum enzymes levels with extract treated animals compared to toxic control animals.

Effect on total bilirubin:

The total bilirubin concentration was found to increase in animals with liver damage by paracetamol .In standard treated group (Silymarin) administration reduced total bilirubin and animal medicaments with EEPD have exhibited dose dependent significant reduction in total bilirubin compared to toxic control group.

Effect on Triglyceride:

Induction of hepatic damage by administration of paracetamol was increase the concentration of triglycerides.treatment with silymarin has shown significantly reduction in the triglycerides levels while EEPD have shown dose dependent decrease in triglyceride level compared to toxic control group**.**

Table 2 Effect of ethanolic extract of pithecellobiumdulce on serum liver profile of Paracetamol induced hepatotoxicity in experimental animals.

Sr. No.	Experimental	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Bilirubn	Triglyceride (mg/dL)
1	Normal Control	54.17 ±1.88	79.67 ± 3.28	103.1± 1.67	0.53 ± 0.029	108.3 ± 2.02
2	Negative Control	149.5 ±1.43	307.4 ±5.07	248.1 ± 1.78 c	2.19 ± 0.028	265.3± 2.56
3	Standard Control	103.5±1.49	119.0 ± 4.69	126.2 ± 0.42	0.92± 0.036	121.2± 1.07
4	Test I	149.0± 1.58	190.5±4.70	191.5± 1.66	1.98± 0.016	197.1± 1.42
5	Test II	143.8± 1.78	175.5± 5.54	169.7± 1.80	1.56± 0.035	165.0± 1.50
6	Test III	110.8± 0.48	149.2± 3.82	147.3± 1.09	0.95± 0.03	134.8± 1.46

Normal control: distilled water; Negative control: Paracetmol: Standard control: Silymarin; Test I: EEPD (100mg/kg); Test II: EEPD (200mg/kg); Test III: EEPD (400mg/kg).

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Values represented mean ± SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. a<0.05, b<0.01, c<0.001; Data compared with normal control; p<0.05, q<0.01, r<0.001 Data compared with negative control; x<0.05, y<0.01, z<0.001 Data compared with standard control (by one way ANOVA followed by Tukey's multiple comparison test).

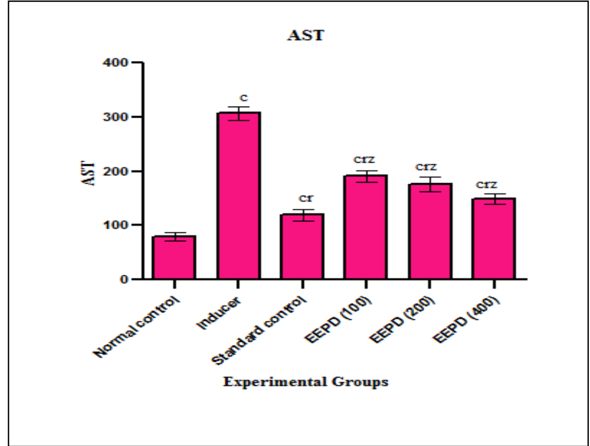

Figure 3: Effect of ethanolic extract of Pithecelobiumdulce on AST (IU/L)

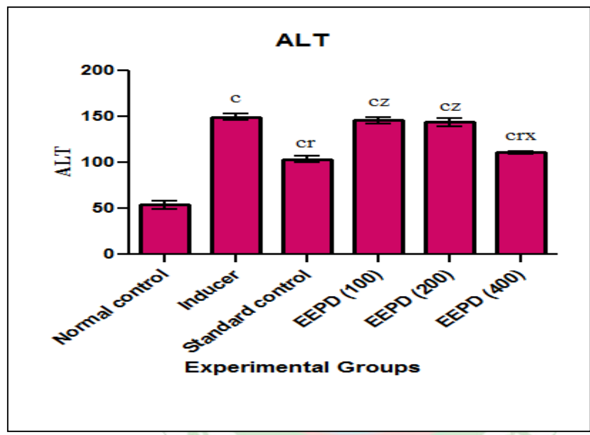

Figure 4: Effect of ethanolic extract of Pithecellobiumdulce on ALT (IU/L)

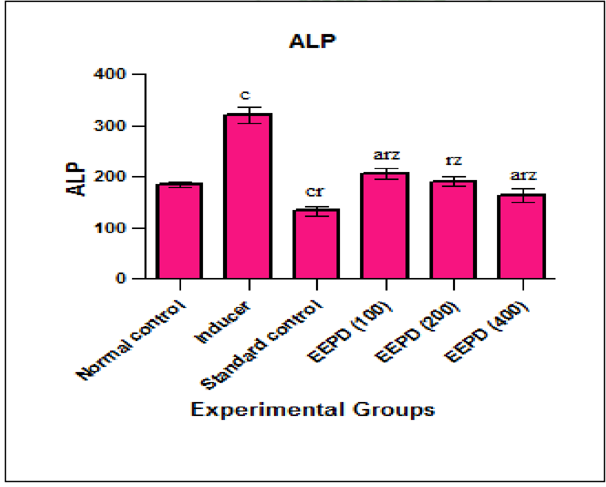

Figure 5: Effect of ethanolic extract of Pithecellobiumdulce on ALP (IU/L)

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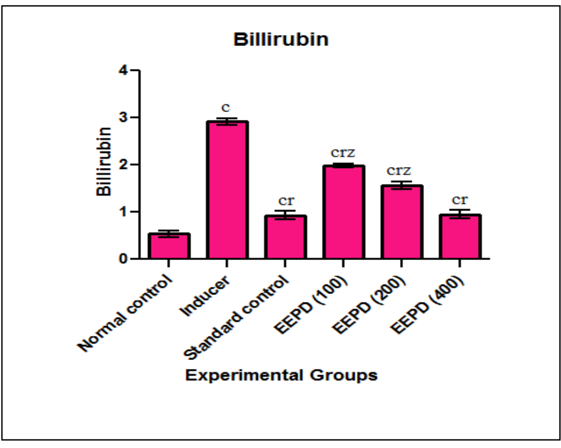

Figure 6: Effect of ethanolic extract of Pithecellobiumdulce on Bilirubin (IU/L)

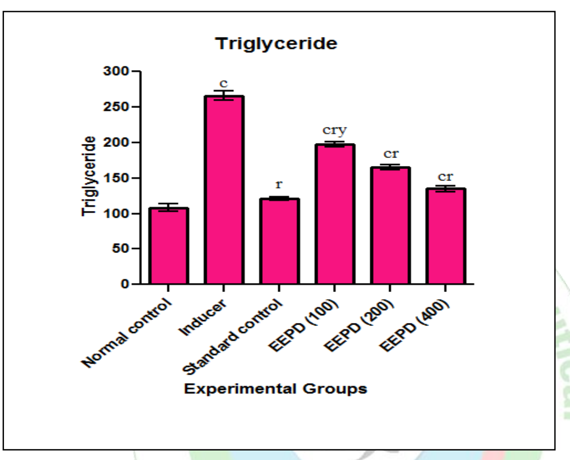

Figure 7: Effect of ethanolic extract of pithecellobiumdulce on triglyceride (mg/dL)

3.4.1. Histopathological observation of liver in normal control group

1.The normal control group animals showed normal hepatocyte architecture such as healthy nucleus and parenchymal structure [Fig.1].

2.Section of liver in negative control group shows partialy effaced architecture compared to normal control group the negative control group animals showed apoptotic changes, perivenular mononuclear inflammatory infiltration, scattered inflammatory infiltration within the parenchyma which is due to toxicity . [Fig.2].

3.When compared with negative control group the test III group animals has reduced fatty changes and restored the hepatocytes near to the normal group [Fig.3].

4.Section of liver in the test drug treated group(200 and 400mg/kg)shows intact architecture few, regenerative hepatocytes, sinusoidal congestion with diffuse congestion of sinusoids.[Fig4,5]

Figure:1 Normal Contol group, showing normal hepatocytes.

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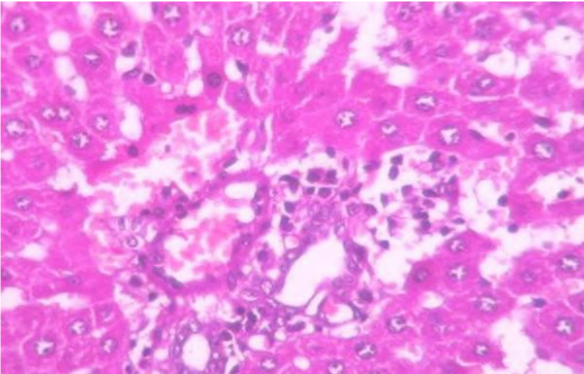

Figure: 2 Paracetamol treated animal group shows that hepatic cells damage And congestion of the liver.

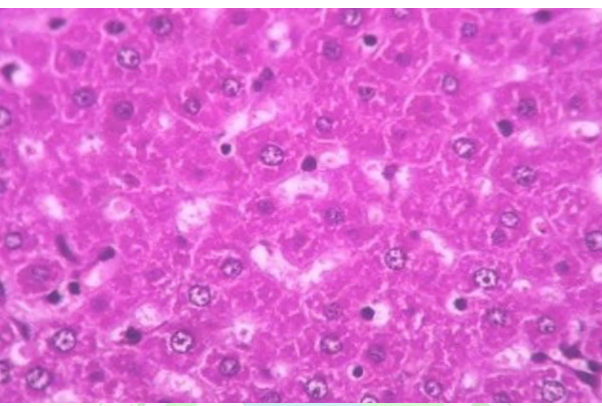

Figure 3: Hepatocytesin grouptreatedwith Standard(Silymarin200mg/kg)

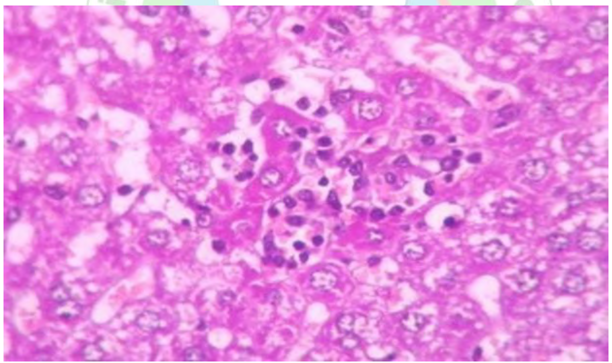

Figure 4: EEPD of 200 mg/kg shows that few regenerative hepatocytes, sinusoidal congestion and scattered mononuclear inflammatory cells

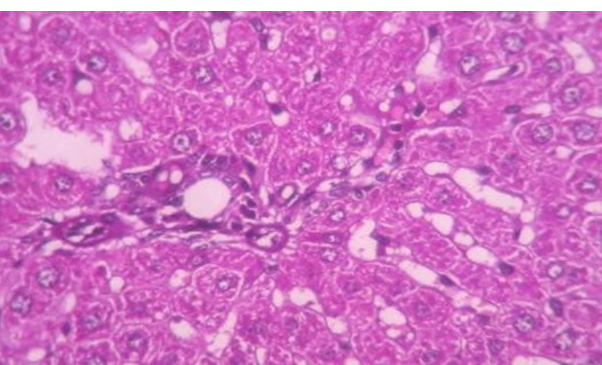

Figure 5: EEPD of 400mg/kg shows that few regenerative hepatocytes ,sinusoidal congestion and scattered mono nuclear inflammatory cells

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5. CONCLUSION

In conclusion, the present study has demonstrated that ethanolic extract of *Pithecellobium dulce* has hepatoprotective effect in paracetamol induced hepatotoxicity. *Pithecellobium dulce* leaves ethanolic extract has showed dose dependent activities on liver weight and volume, various liver functional test Furthermore the better activities has has revealed by the EEPD at dose of 400mg/kg. Utilizing this model,*Pithecellobium dulce* ethanolic extract was showen to be effective in significantly lowering total triglycerides,AST,ALP and ALT levels; thus it can be used in the treatment and/or prevention of liver diseases.

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Role of BDNF in different neurodegenerative diseases

Rohit J Bhadrike, Mahesh M Mali, Bharatee P Chaudhari, Vivekkumar K Redasani
YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Satara, Maharashtra, India

Abstract
Neurodegeneration is the progressive loss of shape or characteristic of neurons, such as death of neurons. Many neurodegenerative diseases consisting of amyotrophic lateral sclerosis, Parkinson's, Alzheimer's, and Huntington's occur due to neurodegenerative processes. Such sicknesses are incurable, resulting in progressive degeneration and loss of life of neuron cells. Neurodegeneration can be discovered in lots of different ranges of neuronal circuitry starting from molecular to systemic Neurodegenerative ailment is an umbrella time period for a range of conditions which on the whole have an effect on the neurons in the human brain. Mind-derived neurotrophic element, additionally called BDNF, is a protein that, in people, is encoded by using the BDNF gene. BDNF is a member of the neurotrophin circle of relatives of increase elements that are associated with the canonical Nerve increase thing. Neurotrophic factors are determined inside the mind and the outer edge. BDNF acts on sure neurons of the valuable apprehensive system and the peripheral nervous machine, helping to support the survival of current neurons, and inspire the increase and differentiation of recent neurons and synapses. Inside the mind, it is energetic inside the hippocampus, cortex, and basal forebrain areas critical to learning, memory, and better wondering. It's also expressed within the retina, motor neurons, the kidneys, saliva, and the prostate. BDNF itself is critical for long-term memory. Even though the massive majority of neurons inside the mammalian mind are shaped prenatally, elements of the grownup mind maintain the potential to grow new neurons from neural stem cells in a procedure known as neurogenesis.

Keywords: neurodegeneration, brain-derived neurotrophic factor, nerve growth factor

Introduction

Brain derived neurotrophic factor (BDNF) is a neuro-defensive protein that regulates neuronal survival, growth and differentiation. The BDNF hypothesis of depression postulates that stress reduces BDNF concentrations in limbic device structures and this underpins the imperative pathogenic process in melancholy, even as antidepressants restore BDNF concentrations and through this alleviate depressive signs and symptoms. This idea has been derived from a wealthy literature and has drawn sizeable assist, so that it will be reviewed on this paper. Further this paper investigates the have an impact on of a not unusual unmarried nucleotide polymorphism (Val66Met) inside the gene encoding BDNF, which has a purposeful position in BDNF expression and can confer susceptibility to melancholy [1].

Brain-derived neurotrophic issue, also known as BDNF, is a secreted protein secreted in humans, is encoded by using the BDNF. Its miles a member of the neutrophin own family of increase factors, NGF. BDNF acts on sure neurons of the vital fearful system and the peripheral fearful gadget, supporting to support the survival of current neurons, and encourage the increase and differentiation of new neurons and synapse. Neurodegeneration is the innovative lack of shape or feature of neurons, including demise of neurons. Many neurodegenerative sicknesses which includes amyotrophic lateral sclerosis, Parkinson's, Alzheimer's, and Huntington's occur because of neurodegenerative methods. Such illnesses are incurable, ensuing in modern degeneration and demise of neuron cells. Neurodegeneration may be located in many distinctive tiers of neuronal circuitry ranging from molecular to systemic Neurodegenerative disease is an umbrella term for a number conditions which by and large affect the neurons inside the human mind. Neurodegenerative disease is an umbrella time period for various conditions which frequently have an effect on the neurons in the human mind. Mind-derived neurotrophic thing, additionally known as BDNF, is a protein that, in human beings, is encoded with the aid of the BDNF gene. BDNF is a member of the neurotrophin circle of relatives of increase factors, which can be related to the canonical Nerve boom factor. Neurotrophic factors are proteins, which play a crucial function in proliferation, differentiation, protection, plasticity, survival and function of neurons within the valuable and peripheral worried systems. These neuroprotective molecules exert sizable manage over the existence and demise pathways in cells. They participate in local responses to diverse types of neuronal stressors. In mammals, the neurotrophin brain-derived neurotrophic issue (BDNF) is a primary regulator of axonal growth and connectivity, neuronal differentiation, survival and synaptic plasticity. It is a key molecular goal in the development of medication in opposition to neurological problems. Several studies have proven the involvement of BDNF within the pathogenesis of neurodegenerative illnesses and psychiatric disorders, like depression and schizophrenia. The neurotrophic movements of BDNF had been installed with various neuronal populations. In the periphery system, BDNF has proven neurotrophic movements on small fiber sensory neurons concerned in sensory neuropathies

Brain Derived Neurotropic Factor (BDNF)

Brain-derived neurotrophic factor, additionally known as BDNF, is a secreted protein secreted in humans, is encoded by using the BDNF. It is a member of the neutrophin own

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family of growth factors, NGF. BDNF acts on positive neurons of the relevant worried system and the peripheral worried device, helping to assist the survival of existing neurons, and inspire the boom and differentiation of new neurons and synapse [4].

Feature of BDNF

BDNF acts on positive neurons of the significant nervous system and the peripheral anxious gadget, supporting to aid the survival of present neurons, and encourage the growth and differentiation of latest neurons and synapses. Inside the mind, it's far active within the hippocampus, cortex, and basal forebrain—regions important to learning, memory, and higher questioning. It's also expressed inside the retina, motor neurons, the kidneys, saliva, and the prostate. BDNF itself is important for long-term reminiscence. Despite the fact that the significant majority of neurons within the mammalian mind are formed prenatally, components of the person mind keep the potential to develop new neurons from neural stem cells in a procedure called neurogenesis. Neurotrophins are proteins that assist to stimulate and control neurogenesis, the ability to make BDNF go through developmental defects inside the brain and sensory anxious system, and normally die quickly after delivery, suggesting that BDNF plays an essential position in regular neural development.

Different vital neurotrophins structurally associated with BDNF include NT-3, NT-four, and NGF [5, 6].

Synthesis and Release

BDNF is made within the endoplasmic reticulum and secreted from dense-core vesicles. It binds carboxypeptidase E (CPE), and the disruption of this binding has been proposed to reason the lack of sorting of BDNF into dense-core vesicles. Different trends consist of sensory neuron losses that affect coordination, balance, hearing, flavor, and respiration. Knockout mice additionally showcase cerebella abnormalities and a boom in the range of sympathetic neurons. sure varieties of physical exercising have been proven too markedly (threefold) boom BDNF synthesis inside the human brain, a phenomenon that's in part accountable for exercise-triggered neurogenesis and upgrades in cognitive function. Niacin seems to up modify BDNF and tropomyosin receptor kinase B (TrkB) expression as properly.

Parkinson's disease (PD)

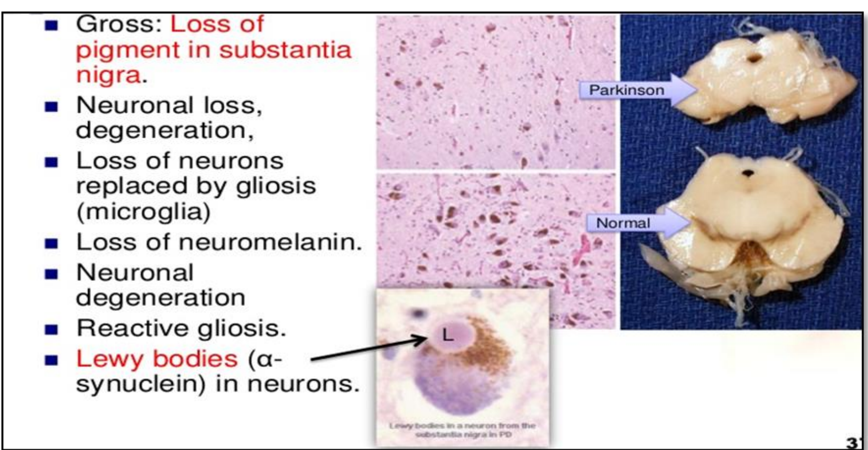

Fig 1: Pathology of Parkinson's diseas

Fig 2: Pathology of Parkinson's disease

Parkinson's disease is basically related to the slow lack of cells inside the substantia nigra of the brain. This region is accountable for the producing of dopamine. Dopamine is a chemical messenger that transmits signals between areas of the mind to coordinate hobby. As an instance, it connects the substantia nigra and the corpus striatum to modify muscle pastime.If there is deficiency of dopamine inside the striatum the nerve cells on this place fire out of manipulate. This leaves the character not able to direct or control moves. This results in the preliminary signs of Parkinson's sickness.

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Because the disease progresses, other regions of the mind and fearful device degenerate as properly causing a more profound motion disease.
A protein known as alpha synuclein seems to be worried in neuronal degeneration. Alpha synuclein is produced thru dopaminergic neurons and is damaged down by using manner of other proteins, which includes parkin and neurosin.
Defects in any of the proteins that smash down alpha synuclein can also result in its accumulation, ensuing in the formation of deposits known as Lewy bodies in the substantia nigra.
However, other mechanisms affecting the accumulation of alpha synuclein had been recognized, and it isn't clear whether or not Lewy bodies are a purpose of or arise as a result of the disorder. Other findings in humans stricken by Parkinson ailment consist of mitochondrial dysfunction, main to accelerated production of free radicals that motive considerable harm to mind cells, and heightened sensitivity of the immune device and neurons to molecules known as cytokines, which stimulate inflammation.

Symptoms
Tremor at relaxation is the characteristic function of PD that earned it the sooner call of the shaking palsy. Rest tremor occurs rarely in every other situation. The tremor is gradual and rhythmic. It normally starts in one hand and handiest later spreads to contain the opposite aspect of initial involvement. Pressure is a term meaning a tightness or boom in muscle tone at relaxation or during the complete variety of movement of a limb. It can be felt as stiffness within the limbs, the neck, or even the trunk. Bradykinsia is slowness in bobbing up or initiating motion, and reduce in high-quality motor coordination (manifested via the inability to button a blouse, cut meat, and so forth.). Gait (walking) lower inside the natural arm swing is visible first, and most effective later do issues with slow, small steps and shuffling (festinating) arise stability issues and impairment of posture usually occur late within the course of normal pd, and are actually the maximum disabling of all the signs.

Treatment
Following drugs used in treatment of Parkinson's disease [10, 11]

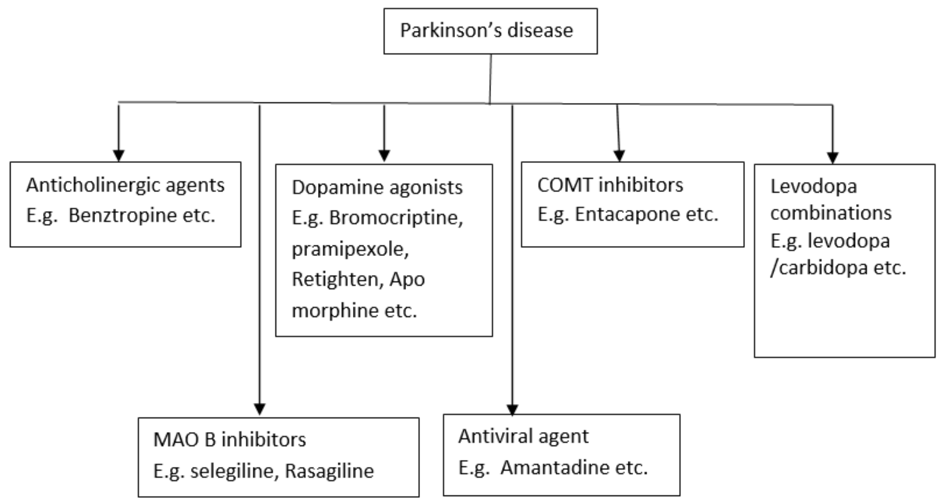

Fig 3: Parkinson's disease

Mechanism of action
Levodopa: Dopamine itself does not go the blood-brain barrier, but it's immediately precursor, levodopa, is with no trouble transported into the CNS and is transformed to dopamine within the brain. Big doses of levodopa are required, because a lot of the drug is decarboxylated to dopamine within the periphery, resulting in aspect outcomes that consist of nausea, vomiting, cardiac arrhyth mias, and hypotension. Carbidopa: The outcomes of levodopa at the CNS can be substantially better through coadministering carbidopa, a dopa decarboxylase inhibitor that doesn't move the blood-brain barrier. Carbidopa diminishes the metabolism of levodopa inside the gastrointestinal (GI) tract and peripheral tissues; for that reason, it increases the provision of levodopa to the CNS. [10].

Function of BDNF in Parkinson's sickness
BDNF performs a position in the advertising of the survival and function of striatal dopaminergic neurons and in regulating synaptic connectivity. other research have shown that BDNF mind and peripheral tiers are decreased in PD patients in comparison to HC verified that remedy with anti parkinsonian pills mayrise BDNF ranges. The position of physical interest in preventing PD onset or development has additionally been verified. BDNF maximum widely expressed and properly characterized member of the neurotropic family within the mammalian mind. It generated following cleavage of the precursor protein proBDNF and BDNF BDNFpro and the prodomuin are all biologically energetic functionally, BDNF has roles in various degree of neuronal in numerous of neuronal circuit development and additionally alter neural citrate shape and synaptic plasticity in person mind in molecular function BDNF in CNS and exceptionally lighting its therapeutic potential for situation which includes Parkinson's ailment stroke and spinal twine injury [12].

Alzheimer's disease (ad)
Alzheimer's disorder is a neurological ailment wherein the loss of life of brain cells reasons reminiscence loss and cognitive decline. A neurodegenerative kind of dementia, the disease starts offevolved moderate and receives gradually worse. [13, 14]

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Pathology

The traditional neuropathological signs and symptoms of Alzheimer's disease are amyloid plaques and neurofibrillary tangles. Plaques consist largely of the protein fragment beta-amyloid. This fragment is made from obvious molecule called amyloid precursor protein. Tangles consist of tau, a protein typically concerned in keeping the inner structure of the nerve cell. at the same time as tau is commonly changed by means of phosphorylation, or the attachment of phosphate molecules, excessive phosphorylation appears to make contributions to tangle formation and stops the protein from sporting out its regular functions. Oxidative stress, or harm to mobile systems by using poisonous oxygen molecules known as free radicals, is also appeared as a pathology characteristic of Alzheimer's. People with Alzheimer's normally revel in brain inflammation. Many of the oldest sufferers with Alzheimer's show symptoms of cerebrovascular disease further to traditional Alzheimer's neuropathology

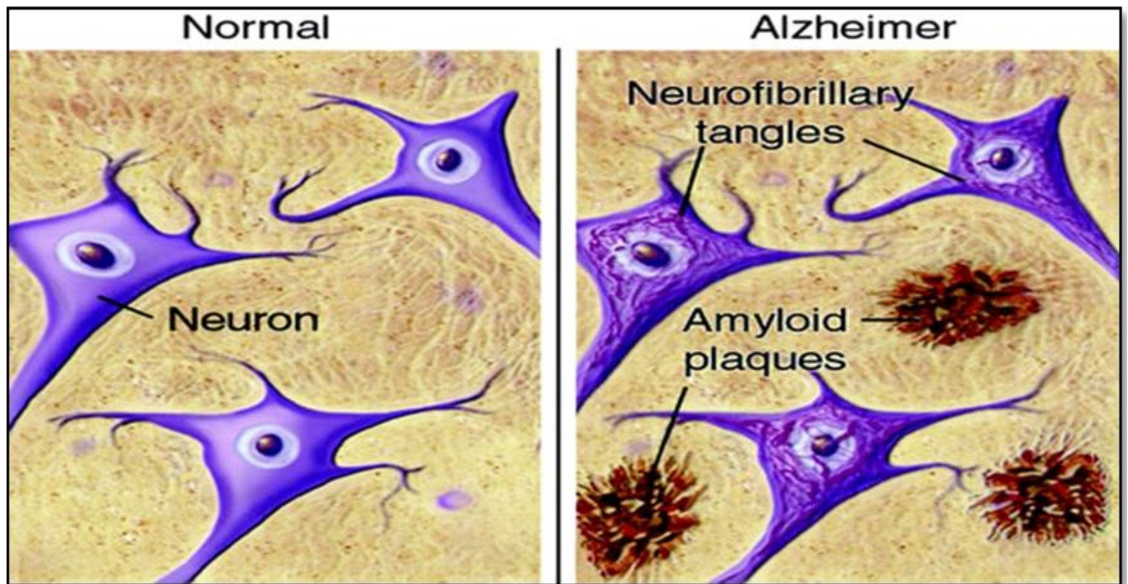

Fig 4: Pathogenesis of Alzheimer's disease

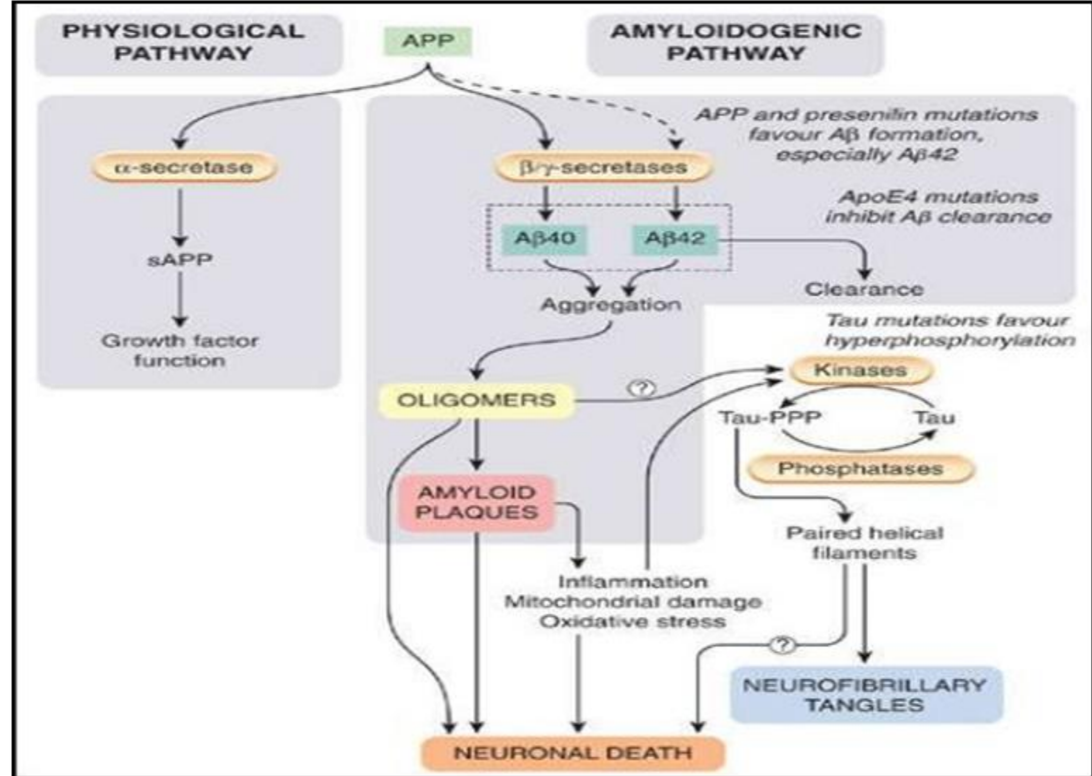

Fig 5: Pathogenesis of Alzheimer's disease

Symptoms

Normal early signs and symptoms of Alzheimer's may also include: reminiscence: often forgetting recent activities, names and faces. Repetition: becoming increasingly more repetitive, e.g. repeating questions after a totally quick interval. Misplacing matters: frequently misplacing objects or setting them in peculiar places. Confusion: Uncertainty approximately the time of day. Disorientation: mainly faraway from everyday surroundings. Language problems: locating the right phrases. Temper and conduct: some human beings turn out to be disinterested in what's going on around them, turn out to be irritable, or lose confidence memory and wondering abilties: humans will discover that their capability to consider, suppose and make selections worsens. Conversation: communique and language come to be tougher. Behavior: a person's conduct might also trade and some people can grow to be unhappy or depressed. Anger and agitation become more not unusual and people may additionally develop anxieties or phobias. Hallucinations: humans can also revel in hallucinations, where they may see matters or human beings that aren't there. Restlessness: problems with dozing and restlessness at night often arise. Unsteadiness: humans may additionally turn out to be increasingly unsteady on their feet and fall more frequently. Each day activities: human beings steadily require extra help with daily sports like: dressing, toileting and consuming.

Treatment

Following drugs used in treatment of Alzheimer's disease [13, 15].

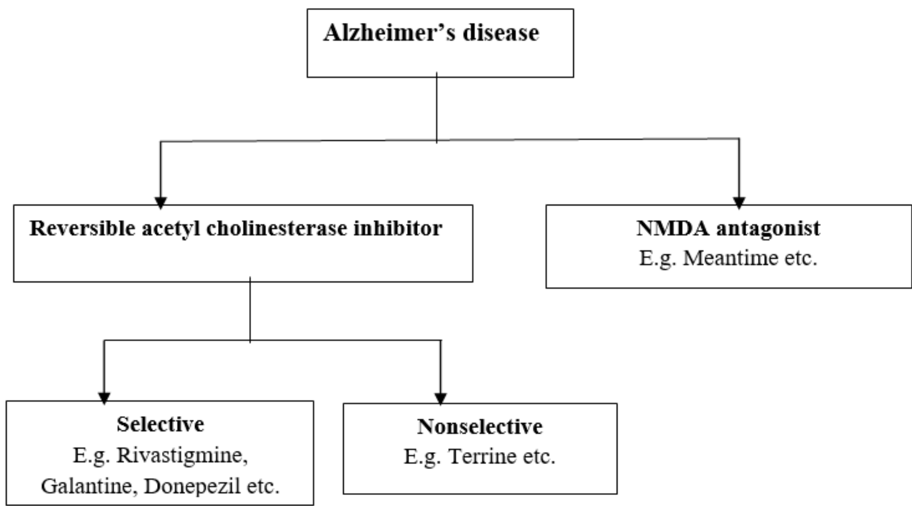

Fig 6: Alzheimer's disease

Mechanism of Action of Memantine

Memantine is the primary in a singular class of Alzheimer's disease medicinal drugs acting at the glutamatergic machine by way of blockading NMDA receptors. It turned into first synthesized by using Eli Lilly and agency in 1968 as a capability agent to treat diabetes; the NMDA interest become found inside the Nineteen Eighties. Memantine is marketed beneath the manufacturers Namenda / Auxura / Ebixa and memory amongst others. Memantine has been proven to have a modest effect in slight-to-extreme Alzheimer's ailment and in dementia with Lewy our bodies. Notwithstanding years of studies, there is little evidence of impact on mild Alzheimer's disease [17].

Role of BDNF in Alzheimer's disease:

It has been suggested that the early reminiscence dysfunction visible in Alzheimer's ailment may be related to the levels of BDNF in the hippocampus. Proof to guide this consists of appreciably decreased BDNF mRNA levels in Alzheimer's sickness hippocampus and parietal cortex and decreased protein levels of BDNF in entorhinal cortex, hippocampus, and temporal, frontal and parietal cortex. In contrast to mature NGF, mature BDNF protein can be visualized by way of western blotting, collectively with its seasoned-form. Both paperwork have now been proven to be decreased in Alzheimer's ailment, with a discount in mature BDNF of 23% reported in frontal cortex moreover a modern lower from everyday turned into visible in proBDNF in MCI (21%), and compared with Alzheimer (30%) parietal cortex [18].

Huntington's ailment

About Huntington sickness is a monogenetic hereditary neurodegenerative sickness caused by a defective gene on chromosome four. the HD gene is liable for generating a protein referred to as Huntington, a protein this is observed during the frame`s tissue however this is most concentrated in the brain. [19].

Pathology

Huntington sickness additionally known as Huntington chorea, an exceedingly rare, and forever deadly, hereditary neurological ailment this is characterised through abnormal and involuntary movements of the muscular tissues and innovative loss of cognitive capability. The disorder became first described by the american medical doctor George Huntington in 1872. Signs and symptoms of Huntington ailment typically seem between the whole of 35 and 50 and get worse over time. They start with occasional jerking or writhing actions, called choreiform movements, or what seem like minor troubles with coordination; these actions, which might be absent all through sleep, get worse over the following couple of years and development to random, uncontrollable, and frequently violent twitchings and jerks. Symptoms of intellectual deterioration may also seem which includes apathy, fatigue, irritability, restlessness, or

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moodiness; these symptoms may progress to memory loss, dementia, bipolar disease, or a toddler of a person with Huntington ailment has a 50 percentage chance of inheriting the genetic mutation related to the sickness, and all folks that inherit the mutation will eventually increase the ailment. The genetic mutation that reasons Huntington disorder happens in a gene known as HD (formally named Huntington [Huntington disease]). This gene, that's located on human chromosome 4, encodes a protein known as Huntington, which is shipped in certain areas of the mind, in addition to different tissues of the body. Mutated kinds of the HD gene include abnormally repeated segments of deoxyribonucleic acid (DNA) referred to as CAG trinucleotide repeats. Those repeated segments bring about the synthesis of huntington proteins that incorporate lengthy stretches of molecules of the acid glutamine. While those odd huntington proteins are reduce into fragments for the duration of processing through cell enzymes, molecules of glutamine challenge out from the ends of the protein fragments, causing the fragments to stick to different proteins. The ensuing clumps of proteins have the ability to purpose neuron (nerve mobile) dysfunction. The formation of strange huntington proteins leads to the degeneration and eventual loss of life of neurons inside the basal ganglia, a couple of nerve clusters deep inside the brain that control motion.

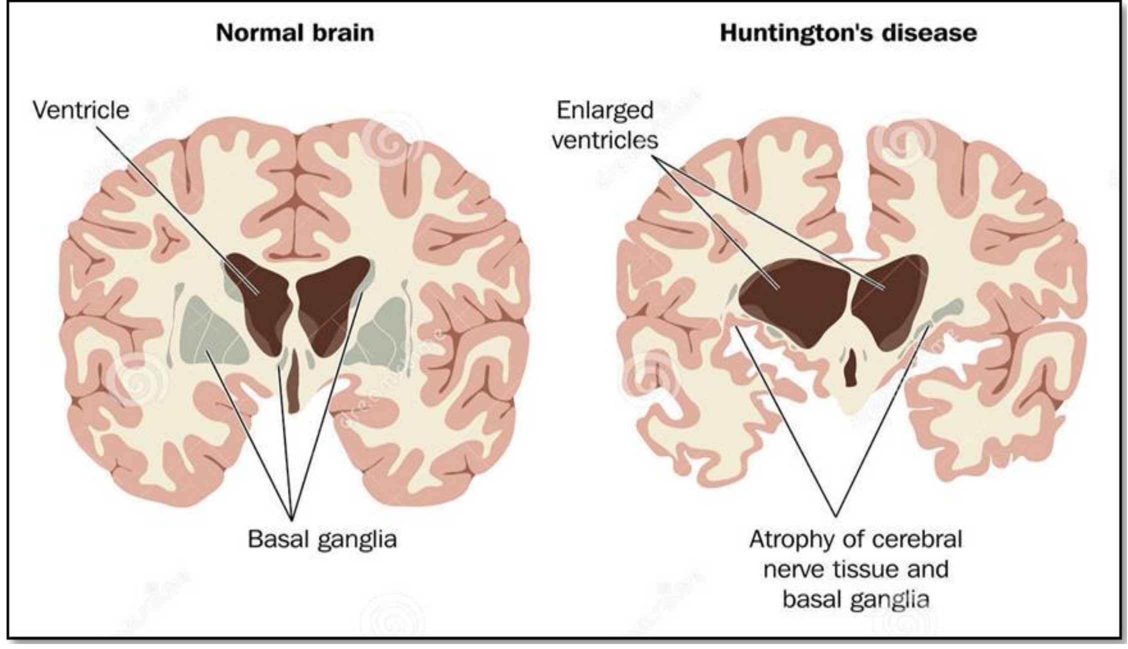

Fig 7: Pathogenesis of Huntington`s Disease

Symptoms

Signs and symptoms can appear at any age, but maximum commonly do so between the 35 and fifty five years. Below is a list of signs which can be relevant in a few instances. it is crucial to do not forget these may also vary depending at the character: moderate uncontrollable actions, Clumsiness, Stumbling, some moderate symptoms of lack of emotion, lack of recognition, moderate attention troubles, Lapses in brief-term memory, melancholy, mood modifications - this can encompass antisocial conduct and aggression [19].

Treatment

Following drugs used in treatment of Huntington Disease

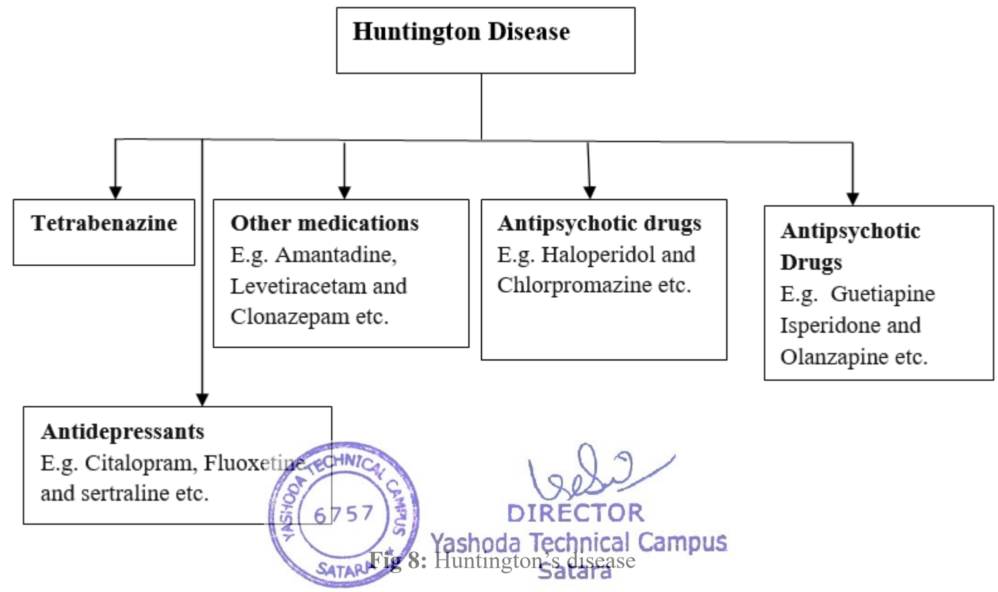

Fig 8: Huntington`s disease

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The Mechanism of Action

Citalopram

The antidepressant, antiobsessive-compulsive, and antibulimic movements of citalopram are presumed to be linked to its inhibition of CNS neuronal uptake of serotonin. Citalopram blocks the reuptake of serotonin on the serotonin reuptake pump of the neuronal membrane, improving the moves of serotonin on 5HT1A autoreceptors. SSRIs bind with considerably less affinity to histamine, acetylcholine, and norepinephrine receptors than tricyclic antidepressant pills [20].

Role of BDNF in Huntington's disease

BDNF performs a function in the mechanism of antidepressant drug motion. The antidepressants known to affect BDNF ranges are selective serotonin reuptake inhibitors (SSRIs) and lithium moreover, memantine, riluzole, (a non-competitive inhibitor of ionotropic glutamate NMDA receptor) cystamine and cysteamine, have lately been proven to growth BDNF levels and their results on HD had been Serotonin might also have defensive consequences on striatal and cortical neurons by using activating cyclic AMP and CREB signals, which additionally cause BDNF expression; different target genes of cyclic AMP-CREB signalling that may play a role within the neuroprotective effect of SSRIs include Bcl-2 and NFKB.

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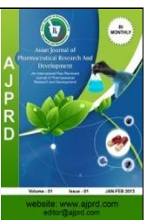

Research Article

Evaluation of Anti-hyperlipidemic Activity of Red Onion In Experimental Animals

Pooja Kadam *[1], Vitthal Chaware [2], Vivek Redasani [3]

[1]Department of Pharmacology, YSPM's Yashoda Technical Campus Wadhe, Satara, India.

[2]Head of Department (Pharmacology), YSPM's Yashoda Technical Campus, Wadhe, Satara, India.

[3]Principal, YSPM's Yashoda Technical Campus, Wadhe, Satara, India.

A B S T R A C T

Objective: To evaluate antihyperlipidemic effect of red onion on poloxamer 407 induced hyperlipidemia in wistar albino rats.

Methods: Hyperlipidemia was induced by intraperitoneal injection of poloxamer-407 (P-407) at a dose of 1.0g/kg body weight in wistar albino rats. Drug treatments were done by oral gavage for 21 days. At the end of the study, animals were kept fasted over night and then blood samples were collected. The serum total cholesterol (TC), triglycerides (TG), and High density lipoprotein (HDL) were measured while low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were calculated by Friedewald formula and atherogenic index was also calculated.

Results: From the present investigation, it was observed that ethanolic extract of red onion have shown significant reduction in serum cholesterol, triglyceride and lipoprotein levels and increase in HDL level in P-407 induced hyperlipidemia.

Conclusion: The findings in this study revealed the effectiveness of ethanolic extract of red onion against hyperlipidemic activity.

Key words: Hyperlipidemia, Poloxamer 407, red onion, Atorvastatin, Lipid Profile

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***Address for Correspondence:**

P. B. Kadam, Department of Pharmacology, YSPM's Yashoda Technical Campus Wadhe, Satara, India.

INTRODUCTION:

Hyperlipidemia is also termed as acquired hyperlipoproteinemia; high blood triglycerides; high blood cholesterol; high cholesterol; high triglycerides; hyperlipidemia etc. [1]. It is an elevation of one or more of the plasma lipids, including triglycerides, cholesterol, cholesterol esters and phospholipids etc. [2]. It is also described by elevation of serum total cholesterol and low density and very low-density lipoprotein cholesterol and decreased high-density lipoprotein levels [3–6]. Number of clinical trials have verified that increase in plasma total cholesterol (TC) and triglycerides (TG) levels are implicated in the development of atherosclerosis [2]. It is also one of the important risk factor for developing cardiovascular diseases (CVDs) [4,7,8]. Principal CVDs associated with hyperlipidemia are hypertension, ischemic heart diseases, stroke, coronary heart diseases (CHDs) and atherosclerosis. They account for almost 80% of the burden of CVD in both developed and developing countries. A 20% decrease in blood cholesterol level can reduce about 31% of CHD incidence, and 33% of its mortality rate [9]. Because of all these risks associated with hyperlipidemia, treatment is often recommended for people with hyperlipidemia [10].

Effective treatment of hyperlipidemia includes dietary modifications and medications. There are number of antihyperlipidemic agents available in the market but they show certain side-effects and contraindications [10]. Statins are the first-line drugs for treatment of hyperlipidemia which act by inhibiting 3-hydroxy-3-methyl-glutaryl-

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coenzyme A (HMGCoA) reductase. However, statins have some adverse effects including rhabdomyolysis and derangements in hepatic function. Fibrates are used as second-line drugs for the treatment of dyslipidemia which acts by activating peroxisome proliferator-activated receptor alpha. However, fibrates shows adverse effects like allergic reactions, nausea, diarrhea etc. Nicotinic acid is also used to treat hyperlipidemia. However, it causes flushing, nausea, vomiting, diarrhea, anorexia like side-effects. Ezetimibe and bile acid sequestrants shows hypolipidemic effect by decreasing intestinal cholesterol absorption; but these drugs are associated with increased gastrointestinal adverse events and also affect the absorption of other biologically important substances [11]. An herbal treatment for hyperlipidemia has no side effects and is relatively less costly, locally available [4]. Hence, people are more interested towards traditional medicinal plants due to their natural origin, safe and non-toxic nature [12].

Red onion (*Allium cepa* L.) is the most widely cultivated species of the genus Allium. The portion of the plant commonly used is the bulb, utilized as a food ingredient to give flavour and aroma to a large variety of dishes [13]. Red onion has been reported to contain flavonoids, phenols, tannins, triterpenoids, cardiac glycosides, saponin and steroid phytochemicals [14]. It also contains Quercetin, cycloalliin, S-methyl-L-cysteine, S-propyl-L-cysteine sulfoxide, dimethyl trisulfide, S-methyl-L-cysteine sulfoxide etc. Literature survey indicates that red onion possesses anti-diabetic [15], Anti-Obesity [16], Hepatoprotective [17], Antidepressant [18], Analgesic [19], Anti-inflammatory [19] and antimicrobial activity etc [14]. Therefore, the present investigation was undertaken to evaluate hypolipidemic effect of ethanolic extract of red onion in poloxamer 407 induced hyperlipidemic rats which has not been previously reported. It is our belief that this investigation will take us another step forward in our quest to understand the mechanism of action of red onion in prevention and treatment of arteriosclerosis and heart related diseases.

MATERIALS AND METHODS:

Drugs & chemical

Poloxamer 407 was acquired from Emcure Pharmaceuticals Ltd Pune. Atorvastatin (Lipvas 20, cipla ltd.) was purchased in a tablet form at strength 20 mg. All other chemicals and reagents used were of analytical grade and procured from approved chemical suppliers. The total cholesterol (TC), triglycerides (TG) and high-density lipoprotein (HDL) were measured with the help of commercial kits.

Collection and extraction of plant material

Fresh red onions were purchased from a local market, satara and were identified by a botanist. They were washed with tap water and then cut into medium pieces. The chopped onions were then blended. The blended onion (200 g) then macerated in 2000 ml ethanol and allowed to stand for a period of 72 h. It was filtered using Whatman filter paper (No. 1). The filtrate was concentrated at 40°C in a water bath for complete dryness. Crude extract obtained was stored at 4°C for further use [20].

Experimental animals

The complete experiment was carried out using 36 wistar albino rats of either sex weighing 150 -200g. The study protocol was approved by Institutional Animal Ethics Committee (IAEC) of YSPM, Satara. The animals were procured from registered breeder and acquainted in the quarantine area for one week. Animals were housed in clean polypropylene cages in a controlled room temperature 22°C ± 2°C, relative humidity of 50 ± 15% and 12 hr dark/ 12 hr light cycle at our Institution's animal house and allowed to acclimatize for two weeks. The animals were fed with standard pellet diet and water *ad libitum*. Animals were maintained as per Committee for the Purpose of Control and Supervision of Experiments on Animals Guidelines.

Preparation of standard drug

Atorvastatin tablets were crushed into powder, dissolved in distilled water at dose 10mg/kg b. w. and administered orally *ad libitum* [9,21].

Induction of hyperlipidaemia

Poloxamer 407 dissolved in cold distilled water at dose 1.0g/kg b. w. and introduced intraperitoneally. All syringes were placed on ice prior to P-407 administration to maintain the polymer in a mobile viscous state during the injection [9,21].

Experimental design

A total 36 wistar albino rats of either sex were randomly divided into 6 groups containing 6 animals in each group. Group 1 (Normal control) did not receive any treatment apart from vehicle 10ml/kg b. w. /day for 21 days. Group 2 (Hyperlipidemic control) were induced with 1.0g/kg b. w. dose of P-407 without treatment [22]. Group 3 (Standard control) were induced with 1.0g/kg dose of P-407 and treated with atorvastatin at a dose of 10mg/kg b. w. /day for 21 days. Group 4, 5 and 6 were induced with 1.0g/kg dose of P-407 and treated with test drug at dose 200 (low), 300 (medium) and 400 (high) mg/kg b. w. /day for 21 days respectively [21].

Blood Sample Collection

At end of the experimental period, animals were kept fasted over night and anaesthetized with diethyl ether. Blood samples were collected serially by retro orbital puncture. The blood was allowed to clot for 30 min at room temperature then serum was separated by centrifugation and used for lipid analysis.

Evaluation parameters

Body weight

Body weight were recorded on the first day of treatment of all groups and final body weight were taken at the end of treatment of all groups to calculate changes between the initial and final body weight of animal throughout the study.

Biochemical parameters

The resulting serum was analyzed for serum TC and TG by Quinoneimine dye absorption method at 505 nm [23] and HDL by precipitation with phosphotungstic acid and Magnesium chloride [24].

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Very low density lipoprotein cholesterol (VLDL-C) was calculated as [11,25,12]:

VLDL = TG/5.

Low density lipoprotein cholesterol (LDL-C) levels were calculated using Friedewald's formula [25]:

LDL = TC - HDL - TG/5

The atherogenic index (A.I.) was calculated using the following formula [26]:

(A.I.) = LOG (TG/HDL)

Liver histopathology

The fixed specimens of liver were processed by washing through running tap water, dehydration through ascending grades of alcohol, clearing through xylene and embedding completely with in paraffin into blocks. The serial sections of not exceeding 3 mm thickness were cut using microtome and were mounted on polylysine coated slides, deparaffinised using xylene, rehydrated and stained with hematoxylin and eosin, dehydrated, cleared and mounted on DPX under glass cover slips. The slides were then observed under light microscope which was connected to a camera to capture images.

Statistical analysis

The results were expressed as Mean ± SEM (n=6). The statistical analysis was carried out with Graph pad prism 5.0 software. The data was statistically analyzed using one-way ANOVA followed by Tukey's multiple comparison tests and $p< 0.05$ was considered to be statistically significant.

RESULTS & DISCUSSION

RESULTS

Yield of the extract

The yield of the extract was found to be 3.7%. Further preliminary phytochemical screening revealed the presence of flavonoids, saponins, phenol, diterpenes, triterpenes, alkaloids, phytosterol and proteins.

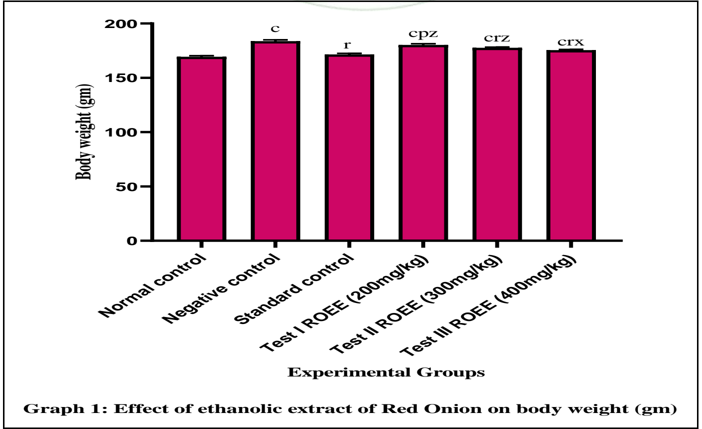
Body weight

Effect of administration of ethanolic extract of red onion on body weight of experimental animals has been shown in table 1. When compared with normal control group, negative control group and all test group animals showed significant ($p<0.001$) increase in body weight but standard control group animals do not show any significant changes in body weight. Furthermore, Standard control group and test II and III group animals showed significant ($p<0.001$) reduction in body weight, whereas test I group showed significant ($p<0.05$) reduction in body weight as compared to negative control group.

Table 1: Effect of ethanolic extract of red onion on body weight of P-407 induced hyperlipidemia in experimental animals.

Sr.	Experimental group	Initial body weight (gm)	Final body weight (gm)	Change in body weight (gm)
1	Normal control	166.6 ± 1.186	169.4 ± 1.622	4.642 ± 1.284
2	Negative control	170.30 ± 3.963	183.8 ± 1.126 ^{c}	13.45 ± 1.098
3	Standard control	168.9 ± 2.032	171.6 ± 1.287 ^{r}	5.33 ± 1.305
4	Test I (200mg/kg)	170.74 ± 2.889	180.3 ± 1.425^{cpz}	9.615 ± 1.491
5	Test II (300mg/kg)	168.75 ± 2.067	177.4 ± 0.689^{crz}	8.03 ± 1.56
6	Test III (400mg/kg)	169.15 ± 1.833	175.5 ± 0.872^{crx}	7.28 ± 1.046

Normal control: distilled water; Negative control: Poloxamer 407; Standard control: Atorvastatin; Test I: ROEE (200mg/kg); Test II: ROEE (300mg/kg); Test III: ROEE (400mg/kg).



Graph 1: Effect of ethanolic extract of Red Onion on body weight (gm)

Values represented mean ± SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. a<0.05, b<0.01, c<0.001; Data compared with normal control; p<0.05, q<0.01, r<0.001 Data compared with negative control; x<0.05, y<0.01, z<0.001 Data compared with standard control (by one way ANOVA followed by Tukey's multiple comparison test)

Serum lipid profile

Poloxamer 407 administration developed acute hyperlipidemia in rats by significantly increasing the level of total cholesterol, triglycerides, lipoproteins and decreasing HDL level as compared to normal control group.

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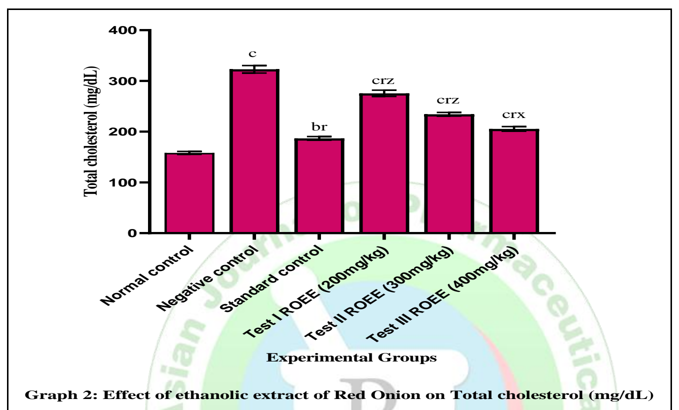
Table 2: Effect of ethanolic extract of red onion on serum lipid profile of P-407 induced hyperlipidemia in experimental animals.

Sr. No.	Experimental group	TC (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	Atherogenic index
1	Normal control	158.3 ± 2.934	105.0 ± 2.061	45.00 ± 1.773	92.25 ± 3.382	21.00 ± 0.4522	0.3691 ± 0.01367
2	Negative control	323.0 ± 7.431^{c}	207.4 ± 5.259^{c}	23.08 ± 0.740^{c}	258.4 ± 6.506^{c}	41.48 ± 1.052^{c}	0.9541 ± 0.01793^{c}
3	Standard control	186.7 ± 3.669^{br}	128.80 ± 3.560^{ar}	38.02 ± 1.003^{br}	123.0 ± 3.420^{cr}	25.76 ± 0.7119^{ar}	0.5298 ± 0.02173^{cr}
4	Test I (200mg/kg)	275.6 ± 6.107^{crz}	185.7 ± 6.401^{cpz}	28.96 ± 0.879^{cpz}	209.5 ± 5.120^{crz}	37.15 ± 1.280^{cpz}	0.8067 ± 0.01864^{crz}
5	Test II (300mg/kg)	234.3 ± 3.614^{crz}	166.3 ± 5.921^{crz}	30.57 ± 1.386^{cqy}	170.40 ± 2.324^{crz}	33.26 ± 1.184^{crz}	0.7364 ± 0.01412^{crz}
6	Test III (400mg/kg)	207.8 ± 4.021^{crx}	150.9 ± 3.912^{crx}	31.55 ± 1.218^{cry}	146.1 ± 4.276^{crx}	30.18 ± 0.782^{crx}	0.6805 ± 0.01656^{crz}

Normal control: distilled water; Negative control: Poloxamer 407; Standard control: Atorvastatin; Test I: ROEE (200mg/kg); Test II: ROEE (300mg/kg); Test III: ROEE (400mg/kg).

Effect of ethanolic extract of red onion on Total cholesterol

Table 2 shows that the ethanolic extract of red onion has significantly affected the level of total cholesterol in hyperlipidemic rats. The results found that the negative control group showed significant (p<0.001) increase in the level of TC as compared to normal control group. Whereas standard control, test I, test II and test III groups showed significant (p<0.001) reduction in TC level when compared with negative control group.

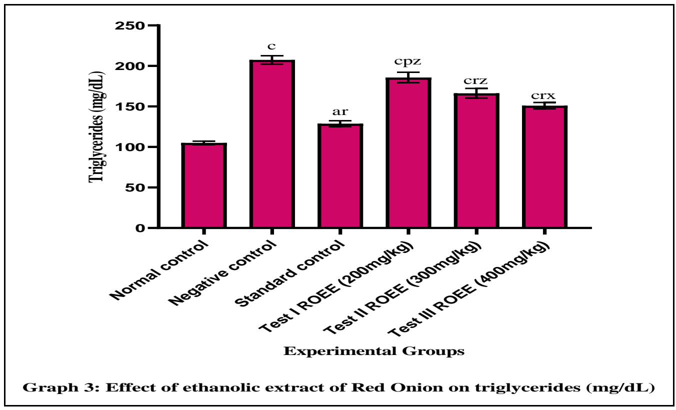


Graph 2: Effect of ethanolic extract of Red Onion on Total cholesterol (mg/dL)

Values represented mean ± SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. a<0.05, b<0.01, c<0.001; Data compared with normal control; p<0.05, q<0.01, r<0.001 Data compared with negative control; x<0.05, y<0.01, z<0.001 Data compared with standard control (by one way ANOVA followed by Tukey's multiple comparison test).

Effect of ethanolic extract of red onion on Triglycerides

The mean values for triglyceride level are given in table 2. Negative control group showed significantly (p<0.001) increased level of triglycerides as compared to normal control group. Standard control, test II and test III group showed significant (p<0.001) reduction and test I group showed significant (p<0.05) reduction in the level of triglycerides as compared to negative control group.



Graph 3: Effect of ethanolic extract of Red Onion on triglycerides (mg/dL)

Values represented mean ± SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. a<0.05, b<0.01, c<0.001; Data compared with normal control; p<0.05, q<0.01, r<0.001 Data compared with negative control; x<0.05, y<0.01, z<0.001 Data compared with standard control (by one way ANOVA followed by Tukey's multiple comparison test).

Effect of ethanolic extract of red onion on HDL

According to the data presented in table 2, the negative control group showed significant (p<0.001) decrease in the level of HDL as compared to normal control group. Whereas significant increase in the level of HDL was observed in standard control (p<0.001), test I (p<0.05), test

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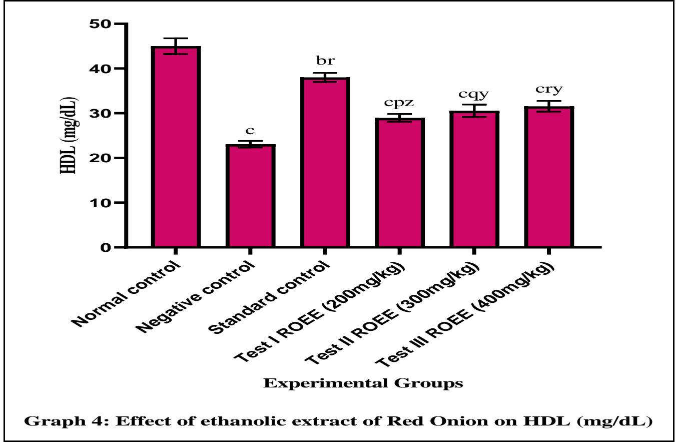
II ($p<0.01$) and test III ($p<0.001$) group when compared with negative control group.

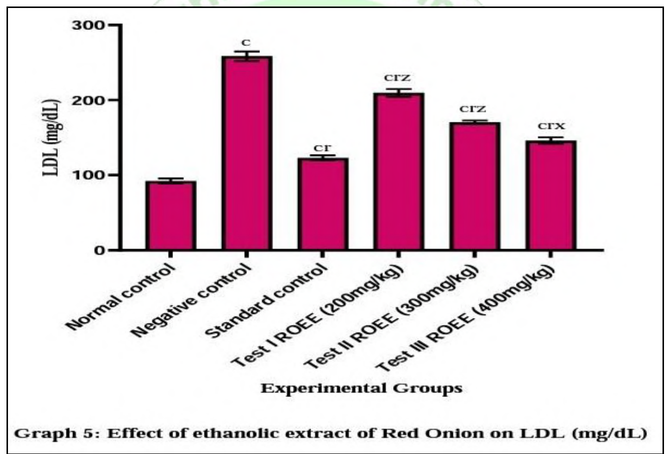


Graph 4: Effect of ethanolic extract of Red Onion on HDL (mg/dL)

Values represented mean ± SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. a<0.05, b<0.01, c<0.001; Data compared with normal control; p<0.05, q<0.01, r<0.001 Data compared with negative control; x<0.05, y<0.01, z<0.001 Data compared with standard control (by one way ANOVA followed by Tukey's multiple comparison test).

Effect of ethanolic extract of red onion on LDL

The mean values for LDL levels in normal and hyperlipidemic groups are given in table 2. When compared with normal control group negative control group showed significant ($p<0.001$) increase in the LDL level. While as compared to negative control group the standard control, test I, II and III group showed significant ($p<0.001$) decrease in LDL level.



Graph 5: Effect of ethanolic extract of Red Onion on LDL (mg/dL)

Values represented mean ± SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. a<0.05, b<0.01, c<0.001; Data compared with normal control; p<0.05, q<0.01, r<0.001 Data compared with negative control; x<0.05, y<0.01, z<0.001 Data compared with standard control (by one way ANOVA followed by Tukey's multiple comparison test).

Effect of ethanolic extract of red onion on VLDL

Table 2 illustrated that the level of VLDL in different treatment groups was considerably affected. The negative control group showed significant ($p<0.001$) increase in the level of VLDL as compared to normal control group. Whereas test I group showed ($p<0.05$) and standard control, test II and III showed ($p<0.001$) significant reduction in VLDL level as compared to negative control group.

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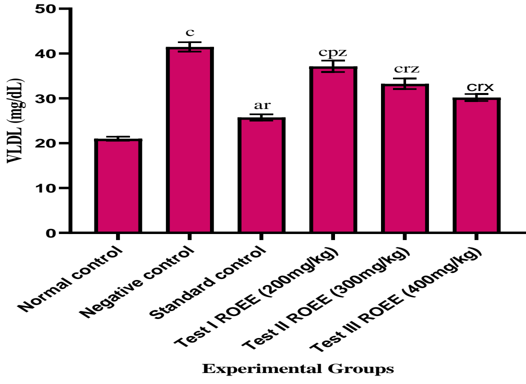
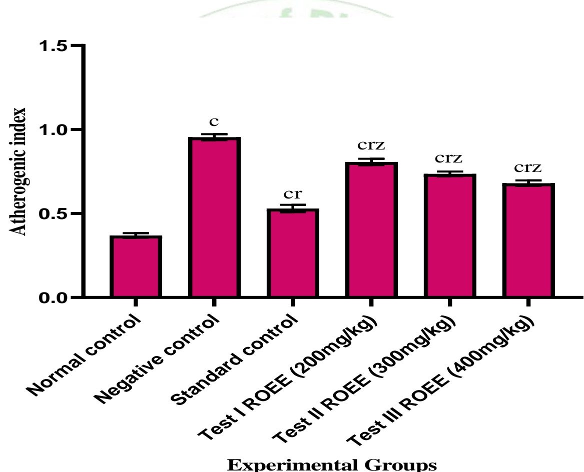


Graph 6: Effect of ethanolic extract of Red Onion on VLDL (mg/dL)

Values represented mean ± SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. a<0.05, b<0.01, c<0.001; Data compared with normal control; p<0.05, q<0.01, r<0.001 Data compared with negative control; x<0.05, y<0.01, z<0.001 Data compared with standard control (by one way ANOVA followed by Tukey's multiple comparison test).

Effect of ethanolic extract of red onion on Atherogenic index

According to the data presented in table 2 the negative control group showed significant (p<0.001) increase in the atherogenic index as compared to normal control group. Whereas standard control and all test groups showed significant (p<0.001) reduction in atherogenic index as compared to negative control group.



Graph 7: Effect of ethanolic extract of Red Onion on atherogenic index

Values represented mean ± SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. a<0.05, b<0.01, c<0.001; Data compared with normal control; p<0.05, q<0.01, r<0.001 Data compared with negative control; x<0.05, y<0.01, z<0.001 Data compared with standard control (by one way ANOVA followed by Tukey's multiple comparison test).

Histopathological changes

Histopathology of the liver for normal control, negative control and test III group were carried out

Histopathological observation of liver in normal control group

The normal control group animals showed normal hepatocyte architecture such as healthy nucleus and parenchymal structure [Fig.1 (a) & (b)].

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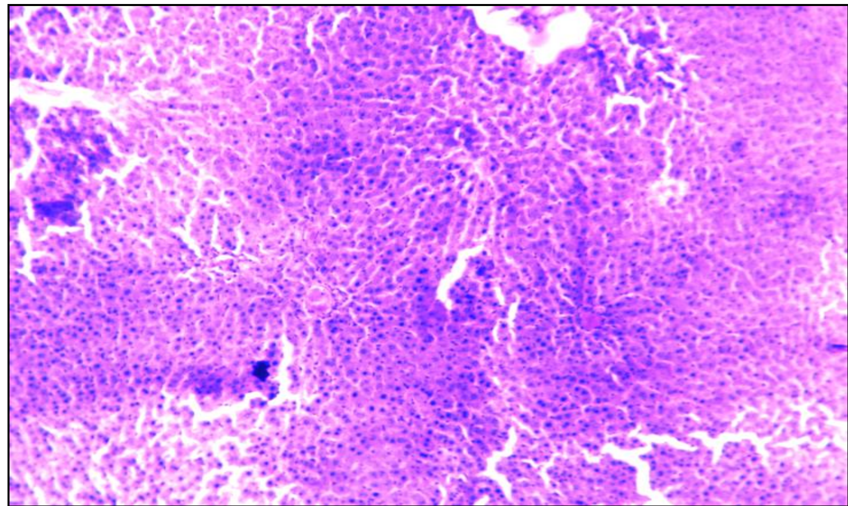

Figure 1 (a): Liver section (100X) of normal control group showing normal hepatocytes

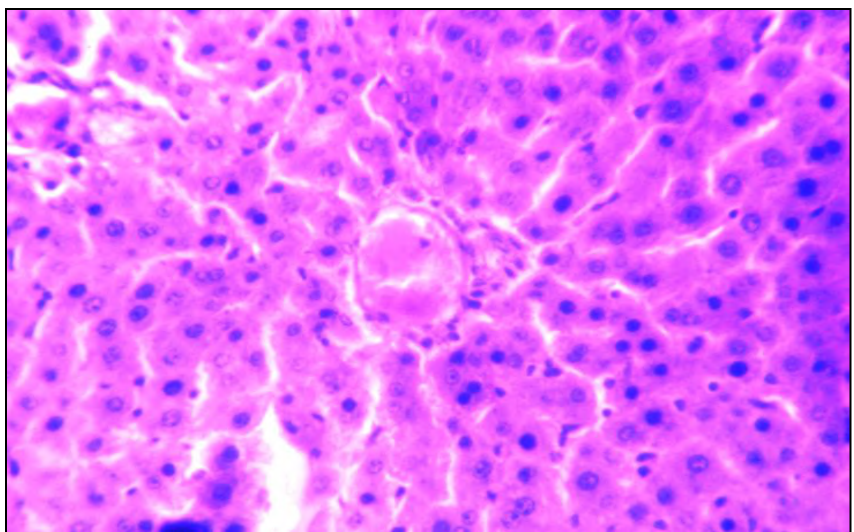

Figure 1 (b): Liver section (400X) of normal control group showing normal hepatocytes

Histopathological observation of liver in negative control group

As compared to normal control group the negative control group animals showed fatty changes, altered hepatocyte architecture along with necrosis, congestion and leucocytic infiltration [Fig.2 (a) & (b)].

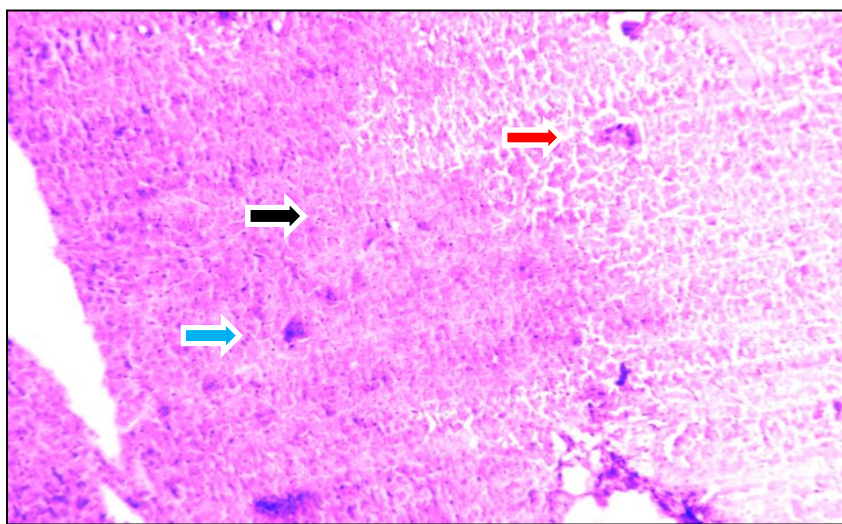

Fig 2 (a). Liver section (100X) of negative control group showing necrosis (Black arrow), congestion (red arrow) and leucocytic infiltration (blue arrow)

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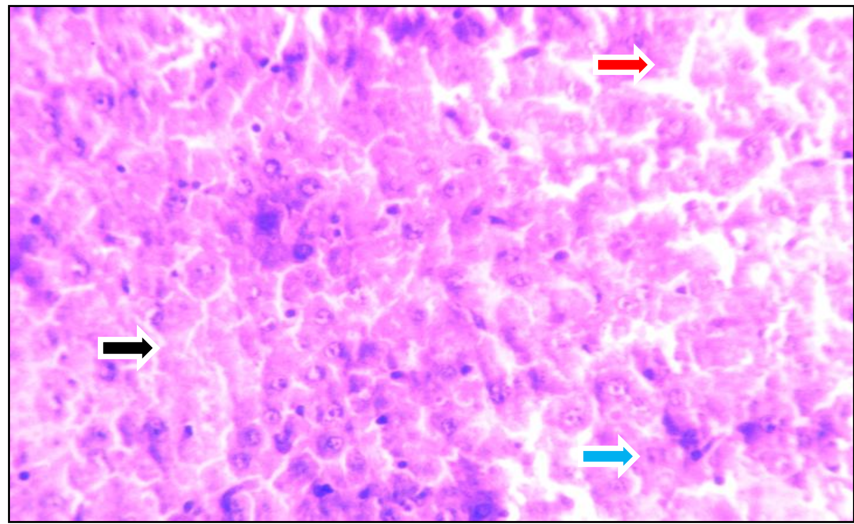

Figure 2 (b). Liver section (400X) of negative control group showing necrosis (Black arrow), congestion (red arrow) and leucocytic infiltration (blue arrow)

Histopathological observation of liver in test III group

When compared with negative control group the test III group animals has reduced fatty changes and restored the hepatocytes near to the normal group [Fig.3 (a) & (b)].

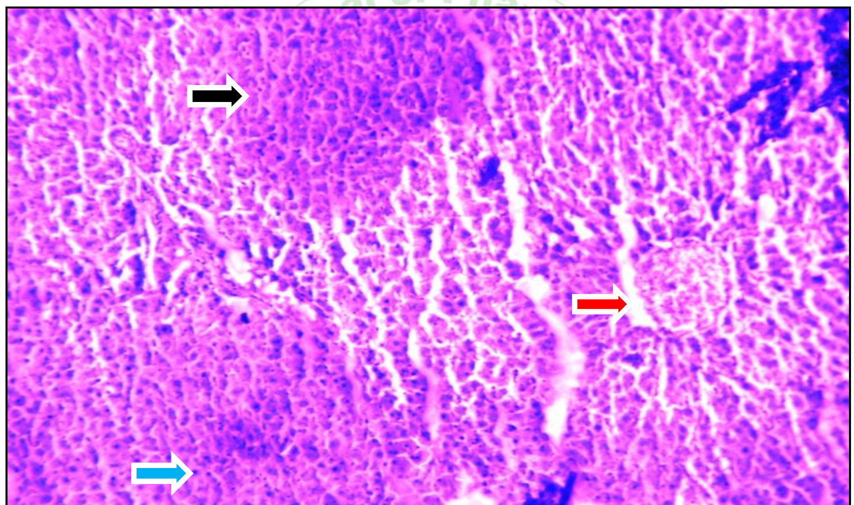

Figure 3 (a): Liver section (100X) of test III group showing necrosis (Black arrow), congestion (red arrow) and leucocytic infiltration (blue arrow)

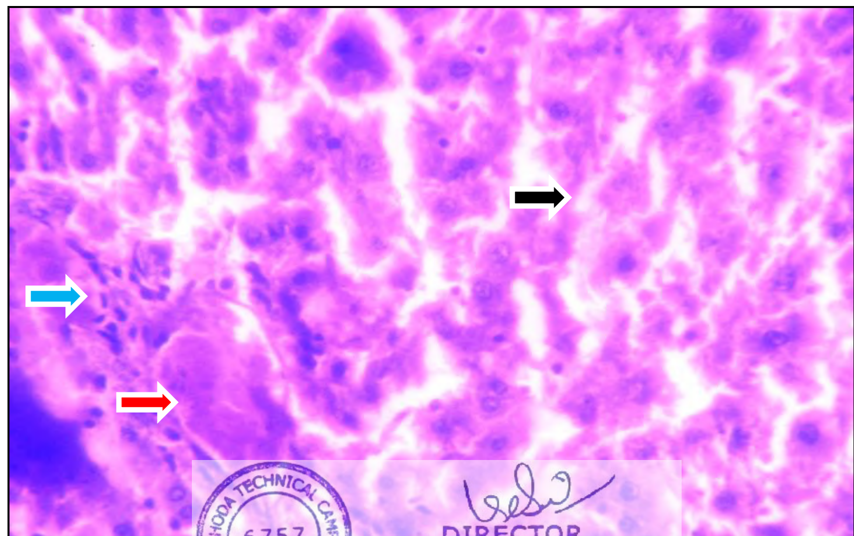

Figure 3 (b): Liver section (400X) of test III group showing necrosis (Black arrow), congestion (red arrow) and leucocytic infiltration (blue arrow)

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DISCUSSION

Lipids are organic compounds which are water insoluble but soluble in organic solvents. Lipids perform number of functions such as chemical messengers, storage and provision of energy, maintenance of temperature and membrane lipid layer formation. Hyperlipidemia is nothing but abnormally elevated level of lipids such as total cholesterol (TC), triglyceride (TG) and lipoproteins [26]. Diseases associated with hyperlipidemia are major risk factors for development of cardiovascular diseases (CVD) [27]. Hyperlipidemia is risk factor for onset and progression of atherosclerosis [28,29] viz high risk factor in development of coronary heart diseases [26]. Hence prevention or treatment of such disorders can be achieved by targeting the causative hyperlipidemia [27].

P – 407 induced hyperlipidemia is one of the animal model used for the evaluation of antihyperlipidemic activity of drug. It was harmless to membranes of cells, in earlier studies it was used effectively to induce hyperlipidemia. Poloxamer 407, a non-ionic synthetic copolymer surfactant commonly known used to induce hyperlipidemia in small laboratory animals within 24 h through i. p. injection. Due to its rapid onset, convenience, reproducibility, and lack of undesirable toxicity, P-407 was used in this study to induce hyperlipidemia in animals [11,30]. A single injection of P-407 caused elevations of serum cholesterol and triglyceride levels in rats. P-407 induced hyperlipidemia via alterations in activity of 3-hydroxy-3- methylglutaryl-CoA (HMG-CoA) reductase, lipoprotein lipase (LPL), lecithin cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP), hepatic lipase (HL) and lipoprotein lipase (LPL). P-407 directly inhibits the capillary (heparin releasable) LPL and HL, and it indirectly increases the biologic activity of CETP and LCAT [9,31].

In our analysis, there was marked increase in the level of total cholesterol, triglycerides, LDL,VLDL, AI and decrease in the level of HDL in negative control group as compared to normal control group (Table 2) confirming that i.p. injection of P-407 has induced hyperlipidemia experimentally [9]. Red Onion ethanolic extract (200mg/kg, 300mg/kg and 400mg/kg) significantly decreased the increased level of total cholesterol, triglycerides, LDL,VLDL, AI and increased the level of HDL after treatment suggest the ameliorative potential of Red Onion.

In our analysis the body weight gain of different groups of rats showed that negative control group animals showed the significant increase in the body weight as compared to normal control group animals. After treatment with standard and test drug the body weight decreased significantly.

In this study, the significant elevation of TC concentration was achieved by the indirect stimulation of HMG CoA reductase by an intraperitoneal (i.p) injection of P-407 [26]. Hence the hypocholesterolemic effect of red onion ethanolic extract could be due to decreased activity of hepatic HMG CoA reductase, stimulation of Cholesterol-7-alpha-hydroxylase, which converts cholesterol into bile acids. It could also be due to the presence of saponins, a phytochemical which forms insoluble complexes with cholesterol or their bile salt precursor, thus making them unavailable for absorption. The results obtained in our analysis conform to earlier report that polyphenols possesses antilipidemic activity [9,26]. Besides, the standard drug (Atorvastatin) used in this study inhibits HMG CoA reductase, viz a rate limiting enzyme in the biosynthesis of cholesterol [26].

In our analysis, elevation in TG concentration after P-407 i.p. injection results primarily from an inhibition of TG degradation, P-407 directly inhibits capillary lipoprotein lipase (LPL) enzyme which is responsible for plasma TG hydrolysis and its clearance from the circulation [11,26]. The Red Onion ethanolic extract could have reduced TG levels by either activating lipoprotein lipase enzyme which hydrolyses the triglyceride into fatty acid hence decreasing triglyceride levels or by inhibiting lipolysis so that fatty acids do not get converted to triglyceride [11, 12, 26].

HDL act as cholesterol scavengers, they transport excess cholesterol and cholesterol esters from the blood and peripheral tissues back to the liver where it is broken down to bile acids. It plays a crucial role in reducing blood and peripheral cholesterol concentrations and inhibits formation of atherosclerotic plaque in the aorta therefore known as the protective cholesterol or Good cholesterol [26]. The present study indicates significant elevation in HDL concentration by the standard drug and ROEE. This could possibly be due to increasing activity of lecithin-cholesterol acyl transferase (LCAT), an enzyme which is responsible for incorporating free cholesterol into HDL there by promoting reverse cholesterol transport and competitively inhibiting the uptake of LDL-c by endothelial cells.

LDL (low density lipoprotein) transports cholesterol to the body cells. It transports near 60-70% of total cholesterol to the body cells. Therefore, an increase in TC level accordingly increases LDL-c [26]. LDL is referred as the most dangerous among the serum lipids, and the oxidation of LDL-c leads to its increased penetration of arterial walls. The increased LDL-c levels play a vital role in the development of atherosclerotic lesions that progress from fatty streaks to ulcerated plaques. Therefore, serum LDL levels are used as the basis for initiating and monitoring the treatment of patients with elevated blood cholesterol levels [9]. In the present study, ROEE shows marked reduction in LDL levels (Table 2). This result could be due to the presence of phenolics, a phytochemical which may work by increasing LDL receptors densities in the liver binding to apolipoprotein B thereby making liver cells more efficient to remove LDL from circulation [26].

Very low density lipoproteins (VLDLs) are secreted from the liver. They contain large amount of triglycerides. As it eventually gets converted into LDL and causes buildup of cholesterol on the walls of arteries it is categorized as a type of bad cholesterol [32]. The present study indicates significant decrease in VLDL concentration by the ROEE. This effect could probably be due to the inhibition of triglyceride and possibly fatty acid synthesis by phenolic constituents of red onion. Atherogenic risk predictor index log (TG/HDL-c) has been considered as the most accurate in determining the extent of atherosclerosis and the risk of myocardial infarction. The present study showed that the Red Onion ethanolic extract has significantly reduced

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atherogenic index as compared to negative control group. The results suggest the anti-atherogenic potential of Red Onion ethanolic extract and hence, reducing the development of coronary atherosclerosis [26].

Histopathology study of liver was also carried out to check fatty changes, necrosis of hepatocytes, congestion and leucocytic infiltration. The histopathological report of negative control group animals showed the development of fatty changes, necrosis of hepatocytes, congestion and leucocytic infiltration while the histopathological report of normal control group animals did not show any fatty changes. Whereas the group of animals treated with ROEE (400mg/kg) restored hepatocytes near to normal control group.

CONCLUSION

In conclusion, the present study has demonstrated that ethanolic extract of Red Onion has antihyperlipidemic effect in Poloxamer 407 induced hyperlipidemia. Red Onion ethanolic extract has showed dose dependent activities on body weight, various serum lipids and atherogenic index. Furthermore the better activities has revealed by the ROEE at dose of 400mg/kg. Utilizing this model, Red Onion ethanolic extract was shown to be effective in significantly lowering total cholesterol, triglycerides, LDL, VLDL and increasing HDL cholesterol levels; also decreasing atherogenic index; thus it can be used in the treatment and/or prevention of cardiovascular diseases.

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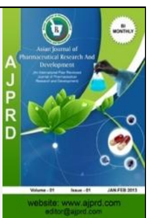

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Research Article

Evaluation of Antidiabetic Potential of *Eucalyptus Globulus* Plant Extract in Dexamethasone-Induced Diabetic Rats

Ujwala B. Kamble*[1], V. J. Chaware[2], V. K. Redasani[3]

[1]Dept. of Pharmacology, YSPM's YTC, Faculty of Pharmacy, Wadhe, Satara, Maharashtra, India.

[2]Head Dept. of Pharmacology, YSPM's, YTC, Faculty of Pharmacy, Wadhe, Satara, Maharashtra, India.

[3] Principal, YSPM's, YTC, Faculty of Pharmacy, NH4 Wadhe, Satara, Maharashtra, India.

ABSTRACT

Objective: Evaluation of Antidiabetic Potential of *Eucalyptus globulus* Plant Extract in Dexamethasone-Induced Diabetic Rats.

Method: Methanolic leaves extract of *Eucalyptus globulus* Plant was prepared by Soxhlet extraction method. Female Albino Wistar rats were made diabetic at the dose of Dexamethasone (5mg/kg/day i.p.) for 12 days. Methanolic leaves extract of *Eucalyptus globulus* Plant (200mg/kg, 400mg/kg & 600mg/kg/day p.o.) was screened for antidiabetic activity. Standard drug Metformin (40mg/kg/day p.o.) was administered to the second group of animals for 12 days. Blood glucose levels and body weights of rats were recorded on 0, 6, 12th days. As well as Hypoglycemic & OGTT evaluation was done. At the end of respective treatment different biochemical estimations & histopathological examination of liver was also carried out.

Result: 12 Days oral administration of the *Eucalyptus globulus* Plant leaves extract caused significant ($P< 0.05$) reduction in blood glucose level & body weight was also gained as compared with toxic control group. Further, it showed the hypoglycemic activity & significant oral glucose tolerance as compared with control animals. The extract also improved other altered biochemical parameters associated with diabetes. Concurrent histopathological examination of liver of these animals showed regeneration by Methanolic leaves extract which was earlier necrosed by Dexamethasone.

Conclusion: Results obtained in this study substantiate the Antidiabetic potential of Methanolic leaves extract of *Eucalyptus globulus* Plant the source of Ellagitannins a bioactive polyphenol and could be considered for further evaluation in clinical studies and drug development.

Keywords: Antidiabetic potential, OGTT, *Eucalyptus globulus*, Polyphenols, Dexamethasone, Insulin.

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***Address for Correspondence:**

Miss. Ujwala B. Kamble, Dept. of Pharmacology, YSPM's, YTC, Faculty of Pharmacy, NH4 Wadhe, Satara, Maharashtra, India.

1. INTRODUCTION

Diabetes mellitus is the chronic metabolic & pancreatic islet disorder mainly characterized by disruption in carbohydrates, protein, and fat metabolism caused by an inability to produce insulin or a defect in utilization. The hyperglycemia caused due to decreased insulin production is called Type-1 diabetes and hyperglycemia due insufficient insulin utilization is called Type-2 diabetes. The feature of diabetes mellitus is polyuria, polydipsia, weight gain and polyphagia. It is also characterized by chronic hyperglycemia and glucosuria, caused by an absolute or relative deficiency of insulin. This may result into the development of further complications which include hypertension, atherosclerosis, ketosis, gangrene and microcirculatory disorders. It is also associated with long-term complications including retinopathy, nephropathy, neuropathy and angiopathy[1]. The IDF (International Diabetes federation) has subsequently released estimates of the numbers of people with diabetes for 2003 and forecasts for 2025 of 194 million and 334 million, respectively. India leads the world

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with largest number of diabetic subjects earning of term “diabetes capital of the world”[2]. Hyperglycemia can be handed initially with oral synthetic 2 Advances in Pharmacological Sciences agent and insulin therapy. Glucose lowering drugs usually succeed in lowering blood sugar levels, therapeutic agents like Insulin, Sulfonylureas, Meglitinides, Biguanides, Thiozolidinediones, DPP-4 inhibitors, α-Glucosidase Inhibitors, Incretin agonists, D2 Agonist may reduce the risk of type 2 diabetes but healthy lifestyle choices remain essential[3]. However, on chronic usage most of these agents produced several side effects including hypoglycemic coma, insulin resistance, hyper-sensitivity, jaundice, abdominal pain, anorexia and metallic taste. Because of the high mortality and morbidity arising from its attendant complications and problems associated with the use of conventional antidiabetic agents[4]. In the natural system of medicine, many Plants have been claimed to be useful for the treatment of diabetes mellitus. The dependence of large rural population on medicinal Plants for treatment of diabetes is because of its availability and affordability. The current worldwide trends towards utilization of Plant-derived natural remedies have, therefore, created a dire need for accurate and up-to-date information on the properties, uses, efficacy, safety, quality & less cost of medicinal Plant products than the semi-synthetics or synthetics. The Plant kingdom has become a target for the search by multinational drug and biologically active lead compounds. In this regard herbal, ayurvedic remedies can improve diabetic conditions without side effects[5]. Ellagitannins (ETs) and ellagic acid (EA) are polyphenols present in some fruits, nuts and seeds, such as pomegranates, black raspberries, raspberries, strawberries, walnuts, almonds & also present in ‘*Eucalyptus globulus* Plant’. Ellagitannins contain various numbers of hexahydroxydiphenoyl units, as well as galloyl units and/or sanguisorboyl units bounded to sugar moiety. In order to determine the quantity of every individual unit, the hydrolysis of the extracts with trifluoroacetic acid in methanol/water system is performed. They form a diverse group of bioactive polyphenols with anti-inflammatory, anticancer, antioxidant and antimicrobial (antibacterial, antifungal and antiviral) activity[6]. So, the present study was undertaken to Evaluate Antidiabetic Potential of *Eucalyptus globulus* Plant Extract the source of Ellagitannins in Dexamethasone - induced Diabetic Rats.

2. MATERIALS & METHODS

2.1 Collection of Plant Material:

Eucalyptus globulus Plant was collected from Local area of Kolhapur District, Maharashtra, India in January 2021 and authenticated as *Eucalyptus globulus (Family: Myrtaceae)* by Department of Botany, Yashwantrao Chavan College of Science, Satara, Maharashtra, India based on the taxonomical features of the whole Plant material including Leaves.

2.2 Preparation of Methanolic Extract:

Leaves of *Eucalyptus globulus* Plant were shade dried for one week after proper cleaning. *Eucalyptus globulus* Plant leaves were coarsely grounded & Methanolic leaves extract was prepared using Soxhlet apparatus by hot percolation method. The obtained extract was concentrated to dryness using rotatory evaporator under reduced pressure & low pressure (<40°C). Extract was kept in air-tight container and stored at 4°C for further studies.

2.3 Phytochemical Screening:

The extract was subjected to phytochemical analysis to test the presence of volatile oils, carbohydrates, alkaloids, glycosides, polyphenols, flavonoids, tannins, propanoids, sterols terpenoids, ketones & alcohols in the leaves extract.

2.4 Drugs and Chemicals:

Dexamethasone obtained from Merck/Dolphin Pharmacy Instruments Pvt. Ltd. Mumbai. Metformin obtained from Aventis Pharma, Ltd. Goa. ACCU-CHECK Active Glucometer procured from Roche Diabetes Care, India/Dolphin Pharmacy Instruments Pvt. Ltd. Mumbai.

2.5 Animals & Housing condition:

Female Albino Wistar Rats of (150-200gm) were selected for experimental study. The animals were maintained under standard laboratory conditions in an animal house approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The animals were kept and maintained under laboratory conditions of temperature 22 ± 2°C, relative humidity 50 ± 15% and 12 hrs. light/dark cycle. They were allowed free access to food (standard pellets) and water ad libitum. Experimental protocols and procedures used in this study was approved by the Institutional Animal Ethics Committee (IAEC) of YSPM’s, YTC, Faculty of Pharmacy, Satara, Maharashtra, India.

2.6 Induction of Diabetes:

Dexamethasone is a synthetic glucocorticoid prevents postoperative nausea and vomiting but may increase blood glucose. These drugs will promote gluconeogenesis or increased blood sugar levels in blood. Chronic exposure to high doses of Dexamethasone causes insulin resistance.

All the Female Albino Wistar animals except control group were administered with Dexamethasone at a dose of 5mg/kg i.p. once a day for 12 days before 1hr. of test drug treatment.

2.7 Collection of Blood samples, Blood Glucose & Body Weight Determination:

Blood samples were withdrawn from tail tip of rats. Fasting blood glucose estimation and body weight measurement were done on 0, 6 & 12th day of the study. Blood glucose estimation can be done by one touch ACCU-CHECK Active Glucometer using glucose test strips.

On day 12th, blood was collected from retro-orbital plexus under mild ether anesthesia from overnight fasted rats and fasting blood sugar was estimated. After that body weight of animals was determined.

2.8 Biochemical Estimation:

Blood samples were with drawn for estimation of Blood glucose level, Serum Insulin level, Lipid profile (Total cholesterol, Triglycerides, HDL, LDL, VLDL) in the serum sample. After the end of respective treatment, animals were sacrificed with high dose of anaesthesia and the tests organs

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were removed, weighed and stored at -20°C for further antioxidant and histopathological studies.

2.9 Experimental Design:

2.9.1 Acute Toxicity Study:

Acute toxicity study was carried out for the *Eucalyptus globulus* Plant by adapting fixed dose method of CPCSEA, OECD guidelines no. 423. Healthy Female Albino Wistar rats were randomly divided into 4 groups with 3 animals in each group. The animals were kept fasted overnight providing only water, after which the Methanolic leaves extract of *Eucalyptus globulus* Plant were administered orally with increasing doses (100, 500, 1000 and 2000mg/kg/day) by intra gastric tube to determine the safe doses by up and down staircase method. The animals were observed continuously for 1 hr., then frequently for 4 hrs. and later at the end of 24 hrs. for general neurological & behavioural or autonomic profile. Further, one group was administered high dose of *Eucalyptus globulus* Plant leaves extract orally once a day for 15 days and observed for any lethality and death.

2.9.2 Hypoglycemic Evaluation:

For Hypoglycemic evaluation, Female Albino Wistar Rats were used and divided into four groups of six animals in each group. Animals were kept fasted overnight (14hrs.) before treatment.

Group I- (Control) rats received vehicle that was 5% Tween 80 (10ml/kg/day p.o.).

Group II- (Test1) rats received Methanolic leaves extract of *Eucalyptus globulus* Plant (200mg/kg/day p.o.) solubilized in 5% Tween 80 solution.

Group III- (Test2) rats received Methanolic leaves extract of *Eucalyptus globulus* Plant (400mg/kg/day p.o.) solubilized in 5% Tween 80 solution.

Group IV- (Test3) rats received Methanolic leaves extract of *Eucalyptus globulus* Plant (600mg/kg/day p.o.) solubilized in 5% Tween 80 solution.

Blood glucose was estimated on 0, 1, 2, 3 & 4th day of the treatment using the ACCU-CHECK Active Glucometer.

2.9.3 Oral Glucose Tolerance Test:

For OGTT evaluation, Female Albino Wistar Rats were used and divided into five groups of six animals in each group. Animals were kept fasted overnight (14hrs.) before treatment.

Group I- (Control) rats received vehicle that was D-Glucose (2gm/kg p.o.).

Group II- (Standard) rats received Glibenclamide (0.5mg/kg i.p.).

Group III- (Test1) rats received Methanolic leaves extract of *Eucalyptus globulus* Plant (200mg/kg p.o.) solubilized in 5% Tween 80 solution.

Group IV- (Test2) rats received Methanolic leaves extract of *Eucalyptus globulus* Plant (400mg/kg p.o.) solubilized in 5% Tween 80 solution.

Group V- (Test3) rats received Methanolic leaves extract of *Eucalyptus globulus* Plant (600mg/kg p.o.) solubilized in 5% Tween 80 solution.

D-glucose (2gm/kg p.o.) was administered to all the rats after one hour of administration of different treatments. Blood glucose was estimated at 30, 60, 90 & 120 min after D-Glucose treatment using the ACCU-CHECK Active Glucometer.

2.9.4 Dexamethasone-Induced Rodent Model of Diabetes:

The Female Albino Wistar rats were divided into six groups of six rats in each. All the animals were fasted overnight (14hrs.) before the treatment of Dexamethasone. All the Female Albino Wistar animals except control group were administered with Dexamethasone at a dose of 5mg/kg i.p. once a day for 12 days. Standard & test drug treatment was started after 1hr. of Dexamethasone administration till the end of study.

Group I- (Control) rats received vehicle that was 5% Tween 80 solution (10ml/kg/day p.o.).

Group II- (Toxic Control) rats received Dexamethasone (5mg/kg/day i.p.).

Group III- (Standard) rats received Metformin (40mg/kg/day p.o.) solubilized in distilled water.

Group IV- (Test1) rats received Methanolic leaves extract of *Eucalyptus globulus* Plant (200mg/kg/day p.o.) solubilized in 5% Tween 80 solution.

Group V- (Test2) rats received Methanolic leaves extract of *Eucalyptus globulus* Plant (400mg/kg/day p.o.) solubilized in 5% Tween 80 solution.

Group VI- (Test3) rats received Methanolic leaves extract of *Eucalyptus globules* Plant (600mg/kg/day p.o.) solubilized in 5% Tween 80 solution.

2.10 Statistical Analysis:

All values of results were presented as mean ± standard error of mean (SEM). The statistical analysis involving two groups was evaluated by means of Student's *t*-test, whereas one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison posttest was used for statistical comparison between control and various treated groups. Statistical significance was accepted at the $p < 0.05$ values.

3. RESULTS

3.1 Quantitative Phytochemical Test:

The yield of extract was found to be 4.8%. The phytochemical analysis revealed that the Methanolic leaves extract of *Eucalyptus globulus* Plant contains a significant amount of volatile oils, carbohydrates, alkaloids, glycosides, polyphenols, flavonoids, tannins, propanoids, sterols and terpenoids, ketones, alcohols.

3.2 Acute Toxicity Study:

In the LD50 value determination, we observed that the *Eucalyptus globulus* Plant extract was safe to use in animals. There was no change in neurological, behavioural

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or autonomic, no lethality or toxic reactions were found with the selected doses (100, 500, 1000 and 2000mg/kg/day p.o.) until the end of study period. Therefore 200, 400 & 600mg/kg was selected for all in-vivo experiments as maximal dose.

3.3 Hypoglycemic Effect of Methanolic Leaves Extract of *Eucalyptus globulus* Plant in Normal Rats:

The results from the study clearly indicated that the administration of Methanolic leaves extract of *Eucalyptus globulus* Plant at the dose 200, 400 and 600mg/kg/day p.o. reduced the blood glucose level significantly on 4th day as compared with normal control group.

Table 1: Hypoglycemic Effect of Methanolic Leaves Extract of *Eucalyptus globulus* Plant in Normal Rats

Sr. no.	Groups (n=6)	Fasting Blood Glucose Level (mg/dl)				
		0th day	1st day	2nd day	3rd day	4th day
I	Control	86.00±0.73	85.16±0.65	83.50±1.05	82.83±1.07	82.33±0.49
II	Test group with Low dose of E. g. Plant leaves extract	86.66±1.05	85.16±0.60	81.00±0.57	79.33±0.33	78.50±0.42*
III	Test group with Intermediate dose of E. g. Plant leaves extract	85.83±1.01	84.83±0.79	81.65±0.91	79.83±0.70	78.62±0.42*
IV	Test group with High dose of E. g. Plant leaves extract	86.16±0.70	83.50±0.67	80.16 ±0.47	78.55±0.67	75.33±0.95*

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnette's multiple comparison test. (*p < 0.05, **p < 0.01).

3.4 Effect of Methanolic Leaves Extract of *Eucalyptus globulus* Plant on the Oral Glucose Tolerance Test in Normal Rats:

The results from the study clearly indicated that the administration of Methanolic leaves extract of *Eucalyptus globulus* Plant at the dose (200, 400 and 600mg/kg p.o.) and Metformin (40mg/kg p.o.) reduced the blood glucose level (hyperglycemia due to glucose load 2g/kg p.o.) significantly after 60 min of administration, as compared with control group.

Table 2: Effect of Methanolic Leaves Extract of *Eucalyptus globulus* Plant on the Oral Glucose Tolerance Test in Normal Rats.

Sr. no.	Groups (n=6)	Fasting Blood Glucose Level (mg/dl) in min				
		0 min	30 min	60 min	90 min	120 min
I	Control	94.17±0.65	98.00±0.74	105.51±1.05	114.84±1.07	121.34±0.49
II	Standard	93.51±0.67	96.17±0.70	89.17±0.47*	88.51±0.67*	85.34±0.95**
III	Test group with Low dose ofE. g. Plant leaves extract	94.17±0.60	96.67±1.05	90.00±0.58	89.34±0.33	88.51±0.42*
IV	Test group with Intermediate dose of E. g. Plant leaves extract	94.84±0.79	95.87±1.01	90.67±0.91	89.84±0.70	88.63±0.42*
V	Test group with High dose of E. g. Plant leaves extract	93.66±1.05	95.74±1.01	89.84±0.70	88.63±0.42	85.94±0.95*

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnette's multiple comparison test. (*p < 0.05, **p < 0.01).

3.5 Effect of Methanolic Leaves Extract of *Eucalyptus globulus* Plant on Body Weight of Diabetic Rats:

At the end of 12 days treatment, body weight was significantly decreased in toxic control group as compared with normal control group & significantly increased in Methanolic leaves extract of *Eucalyptus globulus* Plant at the dose (200, 400 and 600mg/kg/day p.o.) and standard drug Metformin (40mg/kg/day p.o.) treated group as compared with toxic control group.

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Table 3: Effect of Methanolic Leaves Extract of *Eucalyptus globulus* Plant on Body Weight of Diabetic Rats.

Sr. no.	Groups (n=6)	Body Weight of Animals (gm)		
		0 day	**6th day**	**12th day**
I	Control	176.67±0.77	187.33±0.92	193.00±0.92
II	Toxic control	179.17±0.71	184.50±0.57	169.67 ±0.67
III	Standard	179.00±0.78	182.50±1.06**	184.33±0.81**
IV	Test group with Low dose of E. g. Plant leaves extract	174.33±5.05	187.83±0.95**	189.50±0.35**
V	Test group with Intermediate dose of E. g. Plant leaves extract	179.67±0.50	186.33±1.71**	188.33±0.56**
VI	Test group with High dose of E. g. Plant leaves extract	179.67±0.50	183.33±1.71**	186.67±0.77**

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnette's multiple comparison test. ($^{*}p < 0.05$, $^{**}p < 0.01$).

3.6 Effect of Methanolic Leaves Extract of *Eucalyptus globulus* Plant on fasting blood glucose level in Diabetic Rats:

A marked rise in fasting blood glucose level was observed in toxic control group as compared with normal control group. The Methanolic leaves extract of *Eucalyptus globulus* Plant and standard drug Metformin (40mg/kg/day p.o.) treated group which produced a significant reduction in blood glucose level as compared with toxic control group. Methanolic leaves extract of *Eucalyptus globulus* Plant at the dose (200, 400 and 600mg/kg/day p.o.) exhibited a dose dependent significant antidiabetic potential on 6 & 12th days post treatment.

Table 4: Effect ofMethanolic Leaves Extract of *Eucalyptus globulus* Plant on fasting blood glucose level in Diabetic Rats

Sr. no.	Groups (n=6)	Fasting Blood Glucose Level (mg/dl)		
		0th day	**6th day**	**12th day**
I	Control	88.50±2.06	88.33±2.09	90.33±1.88
II	Toxic control	265.00±1.17	297.67±1.21	349.67±4.57
III	Standard	265.67±1.32	221.67±2.83**	115.25±1.52**
IV	Test group with Low dose of E. g. Plant leaves extract	265.83±0.78	256.83±0.78**	146.83±0.78**
V	Test group with Intermediate dose of E. g. Plant leaves extract	266.33±2.64	244.67±2.01**	140.67±2.01**
VI	Test group with High dose of E. g. Plant leaves extract	268.33±2.64	235.67±1.02**	129.67±2.01**

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnette's multiple comparison test. ($^{*}p < 0.05$, $^{**}p < 0.01$).

3.7 Effect of Methanolic Leaves Extract of *Eucalyptus globulus* Plant on Biochemical Parameters in Diabetic Rats

3.7.1 Serum Insulin Level

After 12 days of treatment period it was observed that decreased serum insulin level in toxic control group as compared with normal control group. Animals treated with Methanolic leaves extract of *Eucalyptus globulus* Plant at the dose (200, 400 and 600mg/kg/day p.o.) and standard drug Metformin (40mg/kg/day p.o.) treated group showed a significant increase in the serum insulin level as compared with toxic control group.

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Table 5: Serum Insulin Level

Sr. no.	Groups (n=6)	Serum Insulin Level (*µU/ml*)
I	Control	18.15±0.55
II	Toxic control	7.25±0.31
III	Standard	17.65±0.33**
IV	Test group with Low dose of E. g. Plant leaves extract	12.32±0.37**
V	Test group with Intermediate dose of E. g. Plant leaves extract	13.32±0.34**
VI	Test group with High dose of E. g. Plant leaves extract	16.32±0.39**

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnette's multiple comparison test. (*p < 0.05, **p < 0.01).

3.7.2 Serum Lipid Profile:

After 12 days of treatment period it was observed that increased level of CHL, LDL, VLDL, TG & decreased HDL level in toxic control group as compared with normal control group. Animals treated with Methanolic leaves extract of *Eucalyptus globulus* Plant at the dose (200, 400 and 600mg/kg/day p.o.) and standard drug Metformin (40mg/kg/day p.o.) treated group showed significant reductions in CHL, LDL, VLDL, TG & significant increase in HDL level as compared with toxic control group .

Table 6: Serum Lipid Profile:

Sr. no.	Groups (n=6)	Total Cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	Triglycerides (mg/dl)
I	Control	65.15±0.82	21.82±0.86	14.00±0.35	16.32±0.65	65.49±0.75
II	Toxic control	94.32±0.70	96.32±0.48	8.82±0.29	21.15±0.29	113.32±1.46
III	Standard	68.65±0.65**	35.00±0.72**	17.82±0.46**	18.15±0.29**	75.50±0.75**
IV	Test group with Low dose of E. g. Plant leaves extract	78.15±0.59**	47.32±0.41**	13.75±0.39**	17.65±0.20**	85.75±0.98**
V	Test group with Intermediate dose of E. g. Plant leaves extract	76.82±0.59**	43.49±0.75**	14.82±0.29**	16.55±0.15**	83.69±0.83**
VI	Test group with High dose of E. g. Plant leaves extract	71.82±0.59**	37.49±0.75**	16.88±0.39**	14.45±0.13**	78.50±0.83**

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnette's multiple comparison test. (*p < 0.05, **p < 0.01).

3.7.3 Antioxidant Activity:

3.7.3.1 For Liver:

Diabetes mellitus significantly reduced antioxidant enzymes level of CAT, POD, SOD & GPx. After 12 days of treatment period it was observed that reductions in level of antioxidant enzymes in toxic control group as compared with normal control group. Animals treated with Methanolic leaves extract of *Eucalyptus globulus* Plant at the dose (200, 400 and 600mg/kg/day p.o.) and standard drug Metformin (40mg/kg/day p.o.) showed significant increase in level of antioxidant enzymes like CAT, POD, SOD & GPx as compared with toxic control group.

Table 7: Antioxidant Activity

Sr. no.	Groups (n=6)	CAT (kU/mg Protein)	POD (U/mg Protein)	SOD (U/mg Protein)	GPx (U/mg Protein)
I	Control	9.0±0.21	8.3±0.38	11.4±0.85	99.6±3.5
II	Toxic control	3.3±0.33	4.3±0.46	4.1±0.50	42.0±1.0
III	Standard	7.9±0.33*	7.1±0.34*	9.5±0.33*	88.0±2.0*
IV	Test group with Low dose of E. g. Plant leaves extract	5.2±0.70*	5.6±0.45*	6.8±0.32*	67.5±2.4*
V	Test group with Intermediate dose of E. g. Plant leaves extract	5.9±0.41*	6.0±0.32*	7.5±0.53*	74.0±2.2*
VI	Test group with High dose of E. g. Plant leaves extract	7.1±0.56	6.8±0.71*	8.7±0.66*	86.2±2.7*

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnette's multiple comparison test. (*p < 0.05, **p < 0.01).

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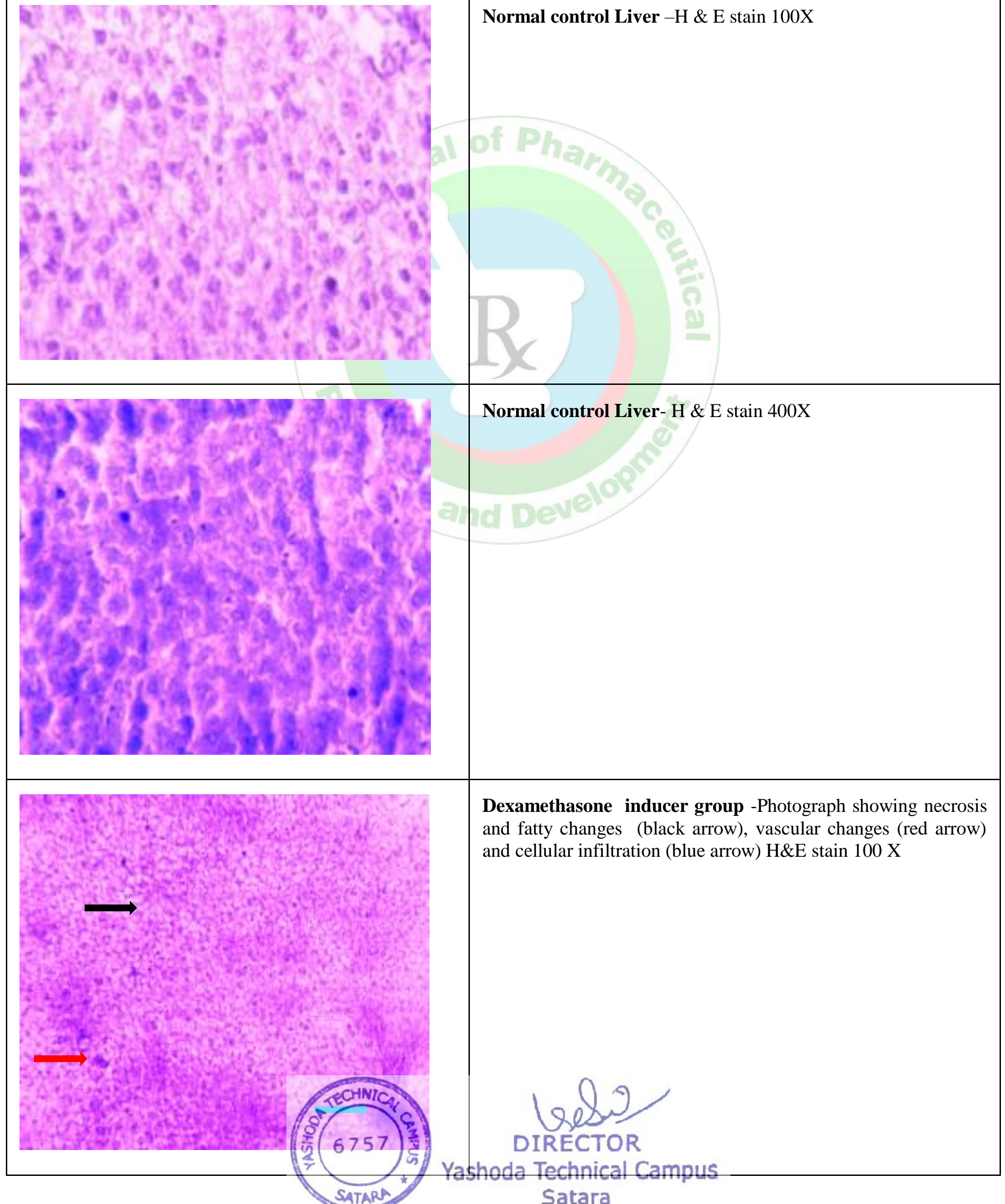
3.8 Histopathological Study:

3.8.1 Liver Histopathology

Table 8: Liver Histopathology

Sr. no.	Group	Necrosis	Cellular changes	Vascular changes
I	Control	0	0	0
II	Toxic control	+++	+++	+++
III	Standard	++	+	0
IV	Test group with Low dose of E. g. Plant leaves extract	+++	++	++
V	Test group with Intermediate dose of E. g. Plant leaves extract	++	++	+
VI	Test group with High dose of E. g. Plant leaves extract	++	+	+



Normal control Liver –H & E stain 100X

Normal control Liver- H & E stain 400X

Dexamethasone inducer group -Photograph showing necrosis and fatty changes (black arrow), vascular changes (red arrow) and cellular infiltration (blue arrow) H&E stain 100 X

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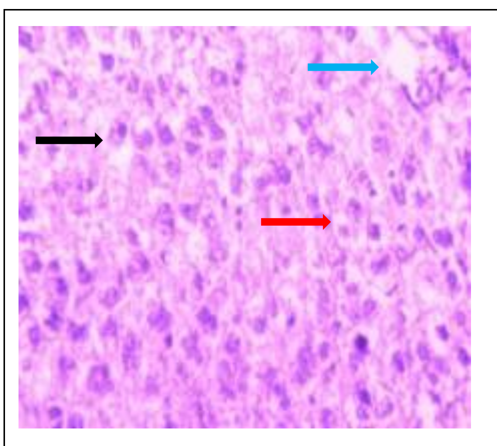
	Dexamethasone inducer group -Photograph showing necrosis and fatty changes (black arrow), vascular changes (red arrow) and cellular infiltration (blue arrow) H & E stain 400 X
	Standard -Photograph showing necrosis and fatty changes (black arrow), vascular changes (red arrow) and cellular infiltration (blue arrow) H & E stain 100 X
	Standard -Photograph showing necrosis and fatty changes (black arrow), vascular changes (red arrow) and cellular infiltration (blue arrow) H&E stain 400 X
	Test – 1 -Photograph showing necrosis and fatty changes (black arrow), vascular changes (red arrow) and cellular infiltration (blue arrow) H & E stain 100 X

	Test – 1 -Photograph showing necrosis and fatty changes (black arrow), vascular changes (red arrow) and cellular infiltration (blue arrow) H & E stain 400 X
	Test – 2 -Photograph showing necrosis and fatty changes (black arrow), vascular changes (red arrow) and cellular infiltration (blue arrow) H & E stain 100 X
	Test – 2 -Photograph showing necrosis and fatty changes (black arrow), vascular changes (red arrow) and cellular infiltration (blue arrow) H&E stain 400 X
	Test – 3 -Photograph showing necrosis and fatty changes (black arrow), vascular changes (red arrow) and cellular infiltration (blue arrow) H & E stain 100 X



Test – 3 -Photograph showing necrosis and fatty changes (black arrow), vascular changes (red arrow) and cellular infiltration (blue arrow) H & E stain 400 X

4. DISCUSSION

4.1 Acute toxicity, Blood glucose level & Body weight Determination:

Globally, the rapid increase the incidence of type 2 DM poses a demand for the quest of novel therapeutic drugs necessitates addition of alternative medicine. As a result number of studies has been conducted to assess the utility of herbal medicine in type 2 DM. The present study was undertaken to evaluate the Antidiabetic Potential of Methanolic Leaves Extract of *Eucalyptus globulus* Plant in Dexamethasone -Induced Diabetic Albino Wistar Rats. In the LD50 value determination, we observed that the *Eucalyptus globulus* Plant extract was safe to use in animals. There was no change in neurological, behavioural or autonomic, no lethality or toxic reactions were found with the selected doses (100, 500, 1000 and 2000mg/kg/day p.o.) until the end of study period. Therefore 200, 400 & 600mg/kg was selected for all in-vivo experiments as maximal dose.

The results of Hypoglycemic study have shown that the Methanolic leaves extract of *Eucalyptus globulus* Plant at the dose 400, 600mg/kg/day has a marked hypoglycemic potential as compared with control group (Table 1).

The Oral glucose tolerance test in normoglycemic rats, blood glucose level was significantly greater in the glucose loaded control group. Methanolic leaves extract of *Eucalyptus globulus* Plant at the dose 200, 400 and 600mg/kgp.o. and Metformin (40mg/kg i.p.) reduced the blood glucose level and improved the impaired glucose tolerance (hyperglycemia due to glucose load 2g/kg p.o.) significantly after 60 min of administration, as compared with control group (Table 2).

Induction of diabetes by Dexamethasone leads to loss of body weight due to increased muscle wasting and loss of tissue proteins, whereas body weight of animals significantly increased in Methanolic leaves extract of *Eucalyptus globulus* Plant at the dose 200, 400 and 600mg/kg/day p.o and standard drug Metformin treated group as compared with toxic control group (Table 3).

Dexamethasone is a synthetic glucocorticoid whose chronic exposure to high doses cause insulin resistance. The results of the antidiabetic study have shown a significant ($p < 0.05$) difference between the 0, 6 & 12th days fasting blood glucose levels of Methanolic leaves extract of *Eucalyptus globulus* Plant at the dose 200, 400 and 600mg/kg/day p.o. and standard drug Metformin (40mg/kg/day i.p.) treated group as compared with toxic control group (Table 4). The possible mechanism of antidiabetic action of Methanolic extract may be by increasing the pancreatic secretion of insulin from the existing beta cells, by its release from the bound form & increase in muscle glucose uptake by increased liver glucose metabolism.

4.2 Biochemical Parameters Analysis:

In Dexamethasone-induced diabetes mellitus showed improvement in biochemical parameters. Methanolic leaves extract of *Eucalyptus globulus* Plant and standard drug treated group showed significant increase in serum insulin level as compared with toxic control group (Table 5). The possible mechanism of action of Methanolic leaves extract may be by increasing the pancreatic secretion of insulin from the existing beta cells, by its release from the bound form.

The serum lipid levels are generally high in diabetes; mapping a major risk factor for coronary heart disease. Chronic exposure to Dexamethasone leads to insulin resistance promotes the increase of hormone sensitive lipase activity. Due to alteration in metabolic parameters leads to an increase in fatty acids mobilizations from adipocytes and increase in hepatic synthesis of triglycerides, which are released into the bloodstream as VLDL, LDL cholesterol. HDL cholesterols enriched with triglycerides which are rapidly hydrolysed and because of their increased catabolism, the blood level of HDL decreases. In a result of lipid profile, marked decrease in total cholesterol, LDL, VLDL and triglycerides was observed, while increase in HDL cholesterol which reduces the risk of atherosclerosis has been observed in Methanolic leaves extract & standard drug treated group which suggest that HDL is inversely related to the total body cholesterol as compared with toxic control group (Table 6). These results could thus reflect the ability of Plant extract improve the tissue sensitivity to insulin. Thus reducing the hormone sensitive lipase activity and increasing the lipoprotein lipase activity, resulting in a decrease of lipolysis these leading to hypolipidemic activity. Flavonoids have been shown to improve dyslipidemia. Thus the hypolipidemic effect of Methanolic leaves extract of *Eucalyptus globulus* Plant could be attribute to the flavonoids contained in the

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Plant. Further these extract could effectively prevent cardiovascular complications related to diabetic dyslipidemia.

In antioxidants study, Diabetes mellitus reduces the antioxidant enzymes level like CAT, POD, SOD & GPx. The increase in the levels of lipid peroxidation might be indicative of a decrease in the enzymatic antioxidant defense mechanism. Animals treated with Methanolic leaves extract of *Eucalyptus globulus* Plant & standard drug treated group showed significant increase in antioxidant enzymes level like CAT, POD, SOD & GPx as compared with toxic control group (Table7).

4.3 Histopathological Examination:

In histopathological study, the fine section of Dexamethasone induced diabetic rats liver on microscopic examination using H & E,100 & 400 X stain showed the normal architecture without steatosis in the normal control group, partial loss of architecture with extensive microvascular steatosis in the toxic control group, normal architecture with microvascular steatosis in the test group i.e. Methanolic leaves extract of *Eucalyptus globulus* Plant treated group, normal architecture with focal microvascular steatosis in the test group i.e. Methanolic leaves extract of *Eucalyptus globulus* Plant treated group, normal architecture without steatosis in the Metformin treated standard group (Table 8).

The results obtained with the Methanolic leaves extract of *Eucalyptus globulus* Plant treatment in chronic diabetic model further clarified the antidiabetic potential of the extract. After 12 days of Methanolic leaves extract of *Eucalyptus globulus* Plant treatment, reductions in elevated blood glucose level,, gain in body weight, hypoglycemic activity, oral glucose tolerance, normalization in altered biochemical parameters & regeneration of damaged tissue of liver were observed, which comparable with that of the toxic control group as well as standard drug Metformin treated group. These effects could be due to the potent bioactive Polyphenol Ellagitannins present in the Plant.

5. CONCLUSION

In conclusion, it can be stated that the Methanolic leaves extract of *Eucalyptus globulus* Plant the source of Ellagitannins has beneficial effects in reducing the elevated blood glucose level as well as gained body weight, hypoglycemic activity, significant oral glucose tolerance, hepatoprotective & normalization in altered biochemical parameters of Dexamethasone-induced diabetic rats. *Eucalyptus globulus* Plant the source of Ellagitannins associated with the stimulation of insulin secretion and enhancement of muscle glucose uptake and metabolism due to regeneration in damaged tissue of liver. These effects could be due to the potent bioactive Polyphenol Ellagitannins present in the Plant. Thus justifying the claim made by ayurvedic classics. Therefore, *Eucalyptus globulus*Plant the source of Ellagitannins represents an effective antidiabetic dietary adjunct for the treatment of diabetes and a potential source for discovery of new orally active agent for future diabetes therapy.

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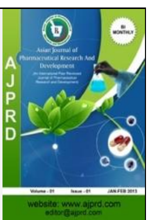
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Research Article

Evaluation of anticataleptic activity of Hydroxytyrosol on Haloperidol induced Catalepsy in Experimental Animal

Mira T. Bansode*[1], V. J. Chaware[2], V. K. Redasani[3]

[1]Dept. of Pharmacology, YSPM's, YTC, Faculty of Pharmacy,Wadhe, Satara, Maharashtra, India.
[2]Head of Dept. (Pharmacology), YSPM's, YTC, Faculty of Pharmacy,Wadhe, Satara, Maharashtra, India.
[3]Principal, YSPM's, YTC, Faculty of Pharmacy, Wadhe, Satara, Maharashtra, India.

A B S T R A C T

Catalepsy is a symptom resulting from problems with the nervous system, and causes muscular rigidity. People with the symptoms may also be less sensitive to touch and have a decreased sensitivity to pain. Catalepsy generally causes people to be unresponsive to speech. It is similar to catatonia, a condition marked by strange movements, lack of movement, and/or general non responsiveness. However, it typically has an underlying physiological cause and does not cause stereotyped movements.

Keywords-Catalepsy, Haloperidol, Scopolamine, Hydroxytyrosol.

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Address for Correspondence:

Mira T. Bansode, Dept. of Pharmacology, YSPM's, YTC, Faculty of Pharmacy, Wadhe, Satara, Maharashtra, India.

INTRODUCTION-

Catalepsy is a condition characterized by inactivity, decreased responsiveness to stimuli and a tendency to maintain an immobile posture. It may be associated with the nervous system drug toxicity, psychotic disorders and other conditions. [1] Catalepsy is the neurodegenerative disease of unknown etiology and characterized by motor symptoms of tremor, rigidity, bradykinesia, and postural instability. Catalepsy is characterized by an abnormal basal ganglia activity. Non-motor comorbidities, such as cognitive impairments (the comorbidity of anxiety and depression like Parkinson's disease) are likely the result of an intricate interplay of multi-system degenerations and neurotransmitter deficiencies extending beyond the loss of dopaminergic nigral neurons. [1][3]

It is an iatrogenic disorder that occurs following chronic anti-psychotic drug treatment that is characterized by motor symptoms dysfunction that is extrapyramidal side effects. When the effect extends beyond the oro-facial region there may be involuntary laryngeal changes and consequent vocalization. Breathing can be affected, as well as the flexion and extension of shoulders, fingers, wrists, hips, knees, ankles and toes. These effects are challenging in social settings, but can also interrupt daily life and personal care. These symptoms are rarely present during sleep and can be halted during attention tasks, however most commonly individuals affected by tardive dyskinesia are not aware of the presence of their symptoms or their ability to voluntarily modulate symptoms. [1][2]

SYMPTOMS OF CATALEPSY

- Extremely rigid body posture
- Decreased sensitivity to pain
- Limbs that stay in the same position when they are moved
- Slower bodily functions
- Particularly breathing
- Decreased muscle control, or complete loss of muscle control (Costall & Naylor, 1974).

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Causes

- Biochemical factor: Low level of neurotransmitter Serotonin, dopamine, norepinephrine.
- Genetic factor: Family member with schizophrenia disorder or psychotic disorder.
- Serious illness: neurological disorders such as Parkinson's disease and epilepsy.
- Substance risk for catalepsy: Medication, substance abuse, drug withdrawal.

MATERIALS AND METHOD-

Animals and housing condition*:*

The experiments will be conducted with Wistar male rats of 110–250 g and 2–3 months old. Female rats are excluded from the present study since estrogen has been reported to possess neuroprotective property and this might mask development of Catalepsy. These animals will be procured from registered breeder and will be acquainted in the quarantine area for one week. After acquaintance, animals will be transferred to the standard laboratory conditions of22±2°Ctemperature,50±15%relativehumidity,12hr.dark/12hr.light)cycleandtheanimals will have free access to pellet diet & water will be provided *ad libitum*. The study protocol will be presented to the IAEC for approval (Dhingra, 2017).

Drug and Reagent- Hydroxytyrosol was acquired from Rajesh Chemicals CO. Mumbai. Scopolamine was obtained from Recipharm Pharmaservices Pvt. Ltd. Bengalore. Haloperidol (serenac 0.25) tablets were procured from RPG Life science Ltd. Gujarat. All other chemicals used were of analytical grade.

Study Design-

The 36 male Albino Wistar rats were divided into six groups: group 1 (control group), group 2 (Catalepsy inducedgroup), group 3 (standard group), groups 4, 5 and 6 (treatment groups). Following Haloperidol administration, standard group was administered with Scopolamine at a dose of 1 mg/kg in oral and treatment groups 4, 5 and 6 were administered Hydroxytyrosol with the dose of 25 mg/kg (Low dose) and 50 mg/kg(Intermediate dose) and 100 mg/kg (High dose) respectively oral for 21 days.

EXPERIMENTAL PROCEDURES

Haloperidol induced catalepsy

A cataleptic behavior will be measured with a high bar test method. Catalepsy score will be measured each hour for 4 h after haloperidol administration, by gently placing both the forepaws of the rat over a metal bar (diameter 2–5 mm) situated 6 cm above the tabletop. The intensity of catalepsy will be assessed by counting the time in seconds until the rat brought both forepaws down to the tabletop, with a maximum cutoff time of 180 s. Finally, scores at different time points (0, 60, 120, 180 and 240 min after haloperidol injection) will be added and expressed as a cumulative catalepsy score for comparison purpose. In all the experiments, the scorer will be blind to the treatment given to the rat. (Naidu & Kulkarni, 2002) (Sanberg et al., 1988).

SCORING OF CATALEPSY –

Cataleptic animal maintaining this position for a period of time dependent upon the degree of catalepsy. If the animal maintained the imposed posture for at least 20s it was said to be cataleptic and given one point.

Scoring will be modified from that used by Costall and Naylor (1974). Animals maintaining the cataleptic posture from 0 s to 10 s scored 0; 10 s to 30 s = 1; 30 s to l min=2; l min to 2 min=3; 2 min to 3 min=4; 3 min to ∞ =5. Animals will be tested for catalepsy 0.5, 1.0, 2.0,

3.0 and 4.0h after haloperidol treatment (Costall & Naylor, 1974)

Statistical Analysis

The statistical analysed by one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test and Dunnett's multiple comparison test. The statistical analyzed by two-way analysis variance followed by Bonferroni Posttests. Data expressed as group mean with standard error mean (mean ± SEM). Data was also compared within group; statistical analysis was done by using paired t-test. P<0.05 was considered to be significant. Statistical analysis and graphical presentation of data was done with Graphed prism-5 software.

RESULTS

Effect of Hydroxytyrosol on catalepsy Score by using High Bar Test

Day 7 : Effect of Hydroxytyrosol on catalepsy activity

Table 1: Effect of Hydroxytyrosol on Catalapsy Score. (7 Day)

Groups	Time (Day 7)				
	30 min	60 min	120 min	180 min	240 min
Normal Control	0.0000±0.0	0.1665 ±0.0	0.0000	0.1666 ±0.0	0.1667 ±0.0
Negative Control	3.168±0.6945***	3.001 ±0.6571***	3.332 ±0.7301***	3.166 ±0.6936	2.668 ±0.5841***
Positive Control	1.500±0.3286**##	1.50 ±0.327**##	1.66 ±0.3651**##	1.833 ±0.4014***#	1.500 ±0.3284***
Low Dose 25mg	2.499±0.5476***	2.668 ±0.5839***	2.834 ±0.6206***	2.332 ±0.5109***	2.334 ±0.5109***
Intermediate Dose 50mg	1.833±0.4014***#	2.333 ±0.5109***	2.333 ±0.5109***#	2.166 ±0.4746***	2.001 ±0.4381
High Dose 100mg	1.832±0.4016***#	2.001 ±0.4381***	2.0001±0.4381***	2.001 ±0.4381***	1.666 ±0.3649

All values are presented as mean ± SEM. Analysis was performed using one way ANOVA followed by Bonferroni multiple comparision posttest. *indicatecomoarision with control group. #indicate comparision of negative control group. @indicate comparision with positive control. */#/@indicate p<0.05, **/##/@@ indicate p<0.01 and ***/###/@@@indicate p<0.001.

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Day 14

Effect of Hydroxytyrosol on catalepsy activity –

Table 2: Effect of Hydroxytyrosol on Catalapsy Score. (14 Day)

Groups	Time (Day 14)				
	30 min	60 min	120 min	180 min	240 min
Normal Control	0.0 ± 0.0	0.0 ±0.0	0.0 ± 0.0	0.0 ±0.0	0.0 ±0.0
Negative Control	2.666 ± 0.6406***	4.0 ± 0.9607***	3.5001 ± 0.8407***	3.332 ± 0.8007***	3.166 ± 0.7607***
Positive Control	1.501 ± 0.3602**#	2.166 ± 0.5205***###	1.832 ± 0.4405***###	1.666 ± 0.4004***###	1.500 ± 0.3604***###
Low Dose 25mg	2.332± 0.5605***	3.001 ± 0.7205***	2.666 ± 0.6406***@	2.501 ± 0.6006***	2.668 ± 0.6404***
Intermediate Dose 50mg	2.000 ± 0.4805***	2.500 ± 0.6005***##	2.333 ± 0.5605***#	2.167 ± 0.5205***#	2.167 ± 0.5205***
High Dose 100mg	1.667 ± 0.4004***###	2.333 ± 0.5605***###	2.000 ± 0.4805***###	1.833 ± 0.4405**##	1.500 ± 0.3604**###

Day 21

Effect of Hydroxytyrosol on catalepsy activity-

Table 3: Effect of Hydroxytyrosol on Catalapsy Score. (21 Day)

Groups	Time (Day 21)				
	30 min	60 min	120 min	180 min	240 min
Normal Control	0.0 ± 0.0	0.0 ±0.0	0.0 ± 0.0	0.0 ±0.0	0.0 ±0.0
Negative Control	1.666 ± 0.4902***	2.666± 0.7355***	3.332 ± 0.9805***	2.666 ± 0.7355***	2.166 ± 0.5882***
Positive Control	0.832 ± 0.2452	1.000± 0.2452###	1.500 ± 0.4412***##	1.167 ± 0.2493*###	1.333 ± 0.3433**
Low Dose 25mg	1.500 ± 0.4412***	2.000 ± 0.5392***@	2.500 ± 0.7355***@	2.332 ± 0.6375***@@	1.882 ± 0.4904***
Intermediate Dose 50mg	1.332 ± 0.3923***	1.833 ± 0.4902**	2.166 ± 0.6375***#	2.166 ± 0.5882***@	1.500 ± 0.3923***
High Dose 100mg	1.333 ± 0.3925***	2.167 ± 0.5883***@@	1.667 ± 0.4903***###	1.667 ± 0.4413***#	1.500 ± 0.3922**

All values are presented as mean ± SEM. Analysis was performed using one way ANOVA followed by Bonferroni multiple comparision posttest. *indicatecomparision with control group. #indicate comparision of negative control group. @indicate comparision with positive control. */#/@indicate p<0.05, **/##/@@ indicate p<0.01 and ***/###/@@@indicate p<0.001.

Effect of Hydroxytyrosol on locomotor activity by using actophotometer

Day 7

Effect of Hydroxytyrosol on locomotor activity

Table 4: Effect of Hydroxytyrosol on Locomotor Activity. (7 Day)

Groups	Locomotor activity counts/10min (Day 7)
Normal Control	482.0 ± 3.917
Negative Control	267.0 ± 1.879***
Positive Control	422.5 ± 1.785***##
HT Low Dose (25mg)	311.5 ± 1.117***##@@
HT Intermediate Dose (50mg)	334.8 ± 2.404***##@@
HT High Dose (100mg)	388.8 ± 2.142***###@@

All values are presented as mean ± SEM. Analysis was performed using one way ANOVA followed by Bonferroni multiple comparision posttest. *indicate comparision with control group. #indicate comparision of negative control group. @indicate comparision with positive control. */#/@indicate p<0.05, **/##/@@ indicate p<0.01 and ***/###/@@@indicate p<0.001.

Day 14

Effect of Hydroxytyrosol on locomotor activity

Table 5: Effect of Hydroxytyrosol on Locomotor Activity. (14 Day)

Groups	Locomotor activity counts/10min (Day 7)
Normal Control	408.7 ± 3.135
Negative Control	192.5 ± 1.196***
Positive Control	433.8 ± 1.726***##
Low Dose 25mg	324.8 ± 1.667***##@@
Intermediate Dose 50mg	346.7± 2.141***##@@
High Dose 100mg	390.8 ± 1.712***###@@

All values are presented as mean ± SEM. Analysis was performed using one way ANOVA followed by Bonferroni multiple comparision posttest. *indicate comparision with control group. #indicate comparision of negative control group. @indicate comparision with positive control. */#/@indicate $p<0.05$, **/##/@@ indicate $p<0.01$ and ***/###/@@@indicate $p<0.001$.

Day 21

Effect of Hydroxytyrosol on locomotor activity

Table 6: Effect of Hydroxytyrosol on Locomotor Activity. (21 Day)

Groups	Locomotor activity counts/10min (Day 7)
Normal Control	468.8 ± 1.077
Negative Control	143.7 ± 2.485***
Positive Control	442.5 ± 3.843***##
Low Dose 25mg	335.6 ± 2.357***##@@@
Intermediate Dose 50mg	360.7 ± 2.954***###@@@
High Dose 100mg	425.3 ± 4.728***###@@

All values are presented as mean ± SEM. Analysis was performed using one way ANOVA followed by Bonferroni multiple comparision posttest. *indicate comparision with control group. #indicate comparision of negative control group. @indicate comparision with positive control. */#/@indicate $p<0.05$, **/##/@@ indicate $p<0.01$ and ***/###/@@@indicate $p<0.001$.

Biochemical Parameter-

Effect of Hydroxytyrosol in Dopamine level (µg/mg)

Effect of Hydroxytyrosol on Dopamine level from rat brain tissue

Table 7: Effect of Hydroxytyrosol on Dopamine Level (µg/mg).

Groups	Dopamine level µg/mg of brain tissue
Normal Control	4.261± 0.24001
Negative Control	1.784 ±0.06501***###
Positive Control	6.574 ± 0.32501***###
Low Dose 25mg	3.501 ± 0.05001*###@@
Intermediate Dose 50mg	4.469 ± 0.3201###@@
High Dose 100mg	6.239 ± 0.6501***###@@

All values are presented as mean ± SEM. Analysis was performed using one way ANOVA followed by Bonferroni multiple comparision posttest. *indicate comparision with control group. #indicate comparision of negative control group. @indicate comparision with positive control. */#/@indicate $p<0.05$, **/##/@@ indicate $p<0.01$ and ***/###/@@@indicate $p<0.001$.

Effect of Hydroxytyrosol in Serotonin level (µg/mg)

Effect of Hydrotyrosol on Serotonin level from rat brain tissue

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Table 8: Effect of Hydroxytyrosol on Serotonin Level (µg/mg).

Groups	Serotonin Level µg/mg of brain tissue
Normal Control	16.9079 ± 0.12500
Negative Control	6.794 ± 0.21500***
Positive Control	19.371 ± 0.31000*###
Low Dose 25mg	12.669 ± 0.34500***###@@@
Intermediate Dose 50mg	14.679 ± 0.31000***##@@@
High Dose 100mg	16.989 ± 0.21500***###@@

All values are presented as mean ± SEM. Analysis was performed using one way ANOVA followed by Bonferroni multiple comparision posttest. *indicate comparision with control group. #indicate comparision of negative control group. @indicate comparision with positive control. */#/@indicate p<0.05, **/##/@@ indicate p<0.01 and ***/###/@@@indicate p<0.001.

Effect of Hydroxytyrosol in SOD level (µg/mg)

Effect of Hydroxytyrosol on SOD level from rat brain tissue

Table 9: Effect of Hydroxytyrosol on SODLevel (µg/mg).

Groups	SOD Level µg/mg of brain tissue
Normal Control	61.231 ± 0.20501
Negative Control	23.179 ± 0.97501***
Positive Control	54.479 ± 0.37001###
Low Dose 25mg	34.979 ± 0.19001***###@@@
Intermediate Dose 50mg	42.659 ± 0.44501***###@@@
High Dose 100mg	52.179 ± 0.29501**###@@

All values are presented as mean ± SEM. Analysis was performed using one way ANOVA followed by Bonferroni multiple comparision posttest. *indicate comparision with control group. #indicate comparision of negative control group. @indicate comparision with positive control. */#/@indicate p<0.05, **/##/@@ indicate p<0.01 and ***/###/@@@indicate p<0.001.

CONCLUSION

In normalizing the various parameters in Catalepsy induced rats, the impact of Hydroxytyrosol therapy with medium (50mg/kg) and high(100mg/kg)was observed to be similar with standard treatment.

In haloperidol induced Catalepsy animals, Hydroxytyrosol recovered the dopamine Serotonin Superoxide dismutase level. Thus, Hydroxytyrosol might be beneficial in managing Catalepsyconditionduetonumerouspharmacologicalactivities includingantioxidant,anticancer,woundhealing,andprotective action against hyperglycemia and hyperlipidemia. By their rich antioxidant activity, Hydroxytyrosol likely promoted protective action against the Catalepsy condition.

Discussion-

Neuroleptics produce two main type of motor disturbances in humans-catalepsy and tardive dyskinesia, collectively called as extrapyramidal side effects, which result directly or indirectly from the blockade of dopamine D2 receptors. These effects constitute the main disadvantages or the therapeutic use of typical neuroleptics. Catalepsy is a characteristic consequence of anti-psychotic drug administration to rats. Most commonly the drug-induced cataleptic effect in rats has been characterized as a reflection of the potential of these drugs to produce extra-pyramidal side effects in humans including Parkinsonianism, akanthesia and dyskinesia.

Haloperidol treatment for 21 successive days significantly induced catalepsy in rats, as indicated by significant increase immobility. This is also supported by the earlier study where haloperidol (1mg/kg) administered once daily in the morning for a period of 21 successive days produced decrease in movements in rats. It has been reported in the literature that chronic use of neuroleptics may lead to imbalance in the production and detoxification of free radicals. Catalepsy is also characterized by accumulation of oxidative damage mainly in the brain due to its high energy metabolism and the relative low activity of antioxidative defence mechanism.

Many factors are proposed for causing catalepsy, such as abnormalities in function of neurotransmitters receptors (e.g., Adenylyl cyclase-cAMP pathway), dysregulation of hypothalamic pituitary adrenal axis (cortisol), changes in the brain monoaminergic transmission (e.g.,5-HydroxyTryptamine, Dopamine, Norepinephrine), increased proinflammatory cytokines (e.g., interleukin-6, tumor necrosis factor-α), increased oxidative stress (e.g., lipid and DNA damage), increased nitric oxide (NO).

In this study, we used two animal models. Actophotometer and high bar test. All the models are widely accepted behavior models for assessing pharmacological anticataleptic activity. All animal were treated with Hydroxytyrosol along with inducer Haloperidol for every model and activity was check on every (i.e., 7th,14th,21th).

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Hydroxytyrosol showed significant anticataleptic activity at doses of 25mg/kg,50mg/kg, and 100mg/kg.

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Yashoda Technical Campus
Satara

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REVIEW ARTICLE

Insilico Molecular docking analysis in Maestro Software

Avanti K. Galande*, Sachin H. Rohane
Department of Pharmacy, Yashoda Technical Campus, Satara- 415015, India.
*Corresponding Author E-mail: **avantigalande7938@gmail.com**

ABSTRACT:
The maestro is the scientific leader in developing state-of-the-art chemical stimulation software for use in pharmaceutical, biotechnology, and, materials science research. Maestro is the portal to all of maestros computational technology-far more than just a user interface, Maestro also helps researches organize and analyz data. Maestro is the culmination of years of research and development. by working closely to be the user environment that is both intuitive and allows our users to get work done efficiently. The typical bacteria form a biofilm which barrier for antibiotics and other harmful substances. The capacity of phages to penetrate that. In the study, we showed that Galloflavin and Ellagic acid increased SIRT6 activity and decreased the expression of SIRT6 associated proteins involved in cancer development. Taken together, Galloflavin and Ellagic acid targeting SIRT6 activity may provide a new insight in the development of anti-cancer therapy. As cetuximab exhibits several anticancer mechanisms, in vivo studies are needed to explore and confirm the effects of combining osimertinib with cetuximab in the L858R/T790M/L792H-mutant pattern.

KEYWORDS: Molecular docking, drug discovery, Maestro software.

1. INTRODUCTION:
Computer-aided drug design uses computational approaches to discover, develop, and analyze drugs and similar biologically active molecules. The ligand-based computer-aided drug discovery (LB-CADD) approach involves the analysis of ligands known to interact with a target of interest. These methods use a set of reference structures collected from compounds known to interact with a target of interest and analyze their 2D or 3D structures. The basic objective of these methods is to predict the nature and strength of binding of given molecule a target. The program renders the rover in a 3D environment. The program features the jet Propulsion Laboratory testing facility. Spirit's landing site to explore. Data from the Spirit and Opportunity's landing site must be downloaded externally from the Maestro website and imported into the program [1-3].

REVIEW OF LITERATURE:
Wieslaw swietnicki and et al used Maestro software to check the in silico analysis of bacteriophage tail tubular suggests a putative sugar binding site and a catalytic mechanism. In silico analysis was performed on the structure of a base tailplate protein gp31 from *Klebsiella pneumonia* bacteriophage KP32 (PDB: 5MU4) which shows activity towards maltose but not trehalose. The first region clearly favored maltose during the docking phase while the second one allowed for the energetically-equivalent binding of trehalose [4].

Carmen Diez-Simon and et al used Maestro software to check the comparison of volatile trapping techniques for the comprehensive analysis of food flavourings by gas chromatography-mass spectrometry. Trapping volatiles is a convenient way to study aroma compounds but it is important to determine which volatile trapping method is most comprehensive in extracting the most relevant aroma components when investigating complex food products. Comprehensiveness and repeatability were compared and SBSE proved particularly suitable for extracting components such as polysulfides, pyrazines and terpene alcohols [5].

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Jae Myung Park and et al used Maestro software to check the A dodecapeptide selected by phage display as a potential theranostic probe for colon cancers. The peptide probe maintained binding affinity even after serum incubation. For therapeutic applications, this peptide probe was conjugated to hematoporphyrin, a photosensitizer, which showed a significantly enhanced cellular uptake and high photodynamic effect to kill tumor cells [6].

K. Wan Yusof and et al used Maestro software to check the developing a UiTM (Perlis) web-based of building space management system: A preliminary study in locating a specified space/room area using open source GIS tool. The preliminary result of the study shows that the spaces and room areas in the building can be mapped out digitaly and it can also be made available to be accessed through the web for the resident of the university [7].

Iman Mirmazloum and et al used Maestro software to check the oxidative stress level and dehydrin gene expression pattern differentiate two contrasting cucumber F1 hybrids under high fertigation treatment. According to RT-qPCR transcript levels of several antioxidant enzymes genes (ascorbate peroxidase, glytathione reductase and glutathione peroxidase) were significantly higher in 'Joker' compared to 'Oitol'. Antioxidant capacity increased in both hybrids with strong characteristics differences favoring 'Oital' plants [8].

Minna Rahnasto-Rilla and et al used Maestro software to check the effects of galloflavin and ellagic acid on sirtuin 6 and its anti-tumorigenic activities. Ellagic acid increased the deacetylase activity of SIRT6 by up to 50-fold; it showed moderate inhibition of SIRT1-3. Galloflavin and ellagic acid showed anti-proliferative effects in Caco2. In this study, we showed that Galloflavin and Ellagic acid increased SIRT6 activity and decreased the expression of SIRT6 associated proteins involved in cancer development. Taken together, Galloflavin and Ellagic acid targeting SIRT6 activity may provide a new insight in the development ofanti-cancer therapy [9].

Susan M. Burden-Gulley and et al used Maestro software to check the A novel molecural diagnostic of glioblastomas: Detection of an extracellular fragment of protein tyrosine phosphate μ[1,2]. The activity of the receptors tyrosine kinase is normally kept in check by the opposing activity of RPTPs such as PTPμ, which are important regulstors of adhesion-dependent signals [10].
H. Bounouria and et al used Maestro software to check the study of heavy metal assessment in the gharb plain along sebou river (morocco) using k0-NAA method triga mark II research reactor. The Ko-mothod activation analysis (ko-NAA) was used in order to determine the concentration of major and trace elements in sediment samples collected from different sites in the Gharb plain aong the Sebou River (morocco). The comparison with other sussequent studies on Sebou River gives an idea about the temporal evolution of heavy metal contamination at national scale [11].

Prasad G. Jamkhande and et al used Maestro software to check the software based approaches for drug designing and development: A systemtic review on commonly used software and its applications. Drug Discovery includes drug designing and development, is a multifarious and expensive endeavor, where least number of drugs that pass the clinical trials makes it to market. Novel software based methods such as molecular modeling; structure-based drug design, structure-based virtual screening, ligand interaction and molecular dynamics are considered to be powerful tool for investigation of pharmacokinetics and pharmacodyanamic properties of drug, and structural activity relationship between ligand and its target [12].

Sejal P. Gandhi and et al used Maestro software to check the computational data og phytoconstituents from *Hibicus rosa-sinensis* on various anti-obesity targets. Molecur docking analysis of twenty two phytoconstitient from *rosa-sinensis*, against seven targets of obesity like pancreatic, hormones as ghrelin, leptin and protein as SCH1and MCH is detailed in this data article. Chemical structures of phytoconstituents were downloaded from PubChem[2][13].

Sabrin R.M. Ibrahim and et al used Maestro software to check the mangostanaxanthone VIIII, a new xanthone from *Garcinia mangostana* pericaps, α-amylase inhibitory activity, and molecular docking studies. The α-amylase inhibitory potential of the isolated metabolites was evaluated. The molecular docking study of the tested metabolites was estimated to shade up therational explanation of the α-amylaseinhibitory activity results [14].

Amin O. Elzupir and et al used Maestro software to check the inhibition of SARS-CoV-2 main protease $3CL^{pro}$ by means of α-ketoamideand pyridine-containing pharmaceuticals usingin silico molecular docking. This study report for the firsttime a compound that could be binding to ALA^{285}, the new residue reaulting from genetic modification of $3CL^{pro}$ of SARS-CoV-2 that hasincresed its catalytic activity 3, 6-fold compare with its predecessor $3CL^{pro}$ of SARA-CoV [15].

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El-Sayed I. El-Desoky and et al Maestro software to check the synthesis, biological evaluation and *in silico* molecular docking of novel 1-hydroxy-naphthyl substituted heterocycles. Chalcone **3c**, naphtyl pyrazoline **6b** and hyadroxycoumarin **13** exhibited the higher activity as antioxidants. *In silico* molecular docking of pyrazoline **6b** endorsed its proper binding at the active site of the 2EX6 enzyme which explains its potent antibacterial activity in comparison with standard ampicillin [16].

Raveendra Melanvanki and et al used Maestro software to check the investigation of interaction between boronic acids and sugar: effect of structural change of sugars on binding affinity using steady state and time resolved fluorecencespectroscopy and molecular docking. Binding interactions of boronic acid derivatives viz. 2-Methylphenylboronic acid (B1) and 3-Methoxyphenylboronic acid (B2) withmono saccharides (arabinose, fructose and galactose) and (sucrose, lactose and maltose) in aqueous condition at pH 7.4 by means of fluorescence spectroscopy is reported in the present investigation [17].

Massound Amanlou and et al used Maestro software to check the anti-HCV anti-malaria agent, potential candidates to repurpose for coronavirus infection: Virtual screening, molecular docking, and molecular dynamics simulation study. Concurrent the viral entranceto the host cell, its antigen will exposure to antigen presentation cells (APC) and then identified by cytotoxic T lymphocytes (CTIs). But reducing the number of CD4and CD8 cells in COVID-19 patients prevents Tcell proliferation and activity [18].

Mohammed M. Matin and et al used Maestro software to check the novel mannopyranoside esters as sterol 14α-demethylase inhibitors: synthesis, PASS prediction, molecular docking, and pharmacokinetic studies. Te activity spectra analysis along with *in vitro* antimicrobial evaluation clearly indicated that those novel MDM estershad better antifungal activitites over antibacterial agents [19].

Andrea Barni and et al used Maestro software to check the Mini-factories for close-to-customer manufacturing of customized furniture: from concept to real demo. During demonstration, customers had the possibility to access the shop, configure their products and see them manufactured in quasi-real time. The promising results of the demonstration activity pave the way for further exploration of the proposed concept [20].

Shobana Sundar and et al used Maestro software to check the Molecular docking, molecular dynamics and MM/PBSA studies of FDA approved drugs for protein kinase a of Mycobacterium tuberculosis; application insights of drug repurposing. Tuberculosis (TB) is a deadly disease, and novel treatment strategies are required to combat it. Repurposing of existing Food and Drug Administration (FDA) approved drugs against Mycobacterium tuberculosis (Mtb) proteinscould be beneficial for TB treatment [21].

CONCLUSION:

After completion of review, it was found that the Meostro molecular docking softwear is very important for Insilico analysis of different pharmaceutical compounds, it aslo helps in determine the molecular interaction, Molecular binding.

This softwear also useful to check the pharmacological activity, therapeutic activities such as Anti tumorigenic activity, Anti HIV activity, Anti malarial activity ETC. After literature review it was seen that numbers of researchers suggest the Meostro softer used for molecular binding, molecular interaction and insilico analytical activity.

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Review Article

Pharmaceutical and biotechnological applications of microsponges as novel nano technological drug delivery system

Shankar B. Kalbhare, Atish B. Velhal, Mandar J. Bhandwalkar, Rupali V. Jadhav, Akash S. Nalawade*

Department of Pharmaceutics, YSPM's, Yashoda Technical Campus, Satara, India 415003

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Abstract

Microsponges drug delivery system composed of porous microsphere. They are tiny sponges-like spherical particles with a larger porous surface. Morever they may enhance stability, reduce side effect and modify drug release favorably. Microsponges technology has many favorable characteristics, which make it a versatile drug delivery system. Microsponge system are based on microscopic, polymer-based microsphere that can suspend or entrap a wide variety of substance, and it can be incorporated into a formulated product such as a gel, cream, liquid or powder. The outer surface is typically porous, allowing a sustained floe of substance out of the sphere. Microsponges are designated to deliver a pharmaceutical active ingredient efficiently at the minimum dose and also to enhance stability, reduce side effect, and modify drug release.

Keywords: Controlled release, Healthcare system, Microsponges, Microsponges Delivery System

Introduction

One of the major challenges in pharmaceutical industry is to control the release of a drug at the specific organ in the body. Now days there are various systems are for targeting the delivery of a drug to a specific organ eg.trasdermal delivery system (Kalbhare et al., 2020). But the transdermal system are not proven for the delivery of the drugs which target the skin. For gastric cancer, there are no systems available which give local effect along with the controlled release of drug. Therefore it is a challenging area for the research work. Microsponges is a type of drug delivery system that enables controlled release and transport of active ingredients too the target organ.

The microsponge drug delivery system was invented by Won in 1987, and the first patent was assigned to Advanced Polymer System. This industry formulated different types of procedures which are applied in the cosmetic and pharmaceutical industry (Jadhav et al., 2013). Microsponge drug delivery systems are polymeric delivery systems composed of porous microspheres. They are small sponge like spherical structures that consist of a countless number of internally connected voids with a larger pores. It consists of non-collapsible structures. Moreover, they increase stability, reduce side effects and transform drug release. Because of the larger porous surface, the drug is released in specific manner. Microsponges have a number of favourable characteristics for targeted drug delivery. Microsponge drug delivery is based on polymeric microscopic spheres that can entrap and suspend wide variety of substances, and thenthey can be incorporated into a formulation such as a cream, gel, or powder. Microsponge drug delivery systemcan increase the efficacy, safety andproduct stability and improve the properties of the formulation in an effective manner (Jadhav et al., 2013; Kaity et al., 2010). Depending upon the size, pore length and pore volume, the microsponge drug delivery system releases the active ingredient.The release of the active ingredient depends on the rubbing, temperature and pH.Microsponges have the ability to absorb the load of polymers and active ingredients in the particles on their surface. Mostly microsponge systems are often used in the transdermal route (Mandavaet al., 2012; Barkai et al., 1990).

***Address for Corresponding Author:**
Akash Shivaji Nalawade
Department of Pharmaceutics,
YSPM's Yashoda Technical Campus,
Satara (India) 415003
Email: kirankal786@gmail.com

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The average size of the microsponges delivery system is in the range 5µm to 300µm in diameter size and a typical 25µm to 250000µm. The surface size of the microsponges varies 20 to 500 µm /g and pore volume range 0.1 to 0.3cm/g. This results in a large reservoir within each microsponge, which can be loaded with up to its own weight of active agent (Jadhav et al., 2013; Kaity et al., 2010; Embil et al., 1996). These pores can entrap large range of drug and other ingredients like emollients, fragrances, essential oils, sunscreens, anti-inflammatory agents. These formulations that can be applied into the targeted region and this entrapped material gets delivered to the skin and controls therelease of the drug.

Potential characters of microsponge

Microsponges are stable at pH range from 1-11 and at high temperatures Microsponges have good compatibility with different type of polymer and ingredients. They also have high entraptment efficiency up to 60-70%. The pore size of microsponges is small so that it prevents the penetration of bacteria. Microsponges does not require sterilization and the addition of preservatives. The system is cost effective and can be used for the long term treatment. The polymeric design of the microsponges is mainly utilized for the controlling the drug release for given period of time and also being used for targeting specific region.

Benefits of microsponges

The microsponges can enhance product performance and also extend the release of drug upto 12 hours. They reduce irritation, increase patient compliance and improve product elegance. Microsponges increase the physical, chemical, thermal stability of drugs and absorb the oil upto 6 times their weight. Because of flexibility of microsponges they can act as novel drug delivery systems. Microsponges are non-irritating, non-mutagenic, non-allergenic and non-toxi. Microsponges allow the incorporation of immiscible products.Microsponges can improve bioavailability of some drugs.

Method of preparation of microsponge

Preparation of Microsponges involves two steps which are liquid-liquid suspension polymerization and quasi emulsion solvent diffusion techniques or w/o/w emulsion technique that can be based on physico chemical properties of drug.

Liquid-liquid suspension polymerization technique

The porous polymeric microspheres can be prepared by liquid-liquid suspension polymerization method. In this method, immiscible polymers are first dissolved with active moities in a suitable solvent. The aqueous phase consist of additives like surfactant, suspending agents to form of suspension. The polymerization process is activated by increasing the temperature. Following this process, the development of reservoir system contributes to the formation of the porous structure. The solvent is then removed and the spherical porous structured microspheres are formed. These formed microspheres are known as microsponges (Burton et al., 2002; Chardeet al., 2013). If the drug is not suitable for the one step procedure mentioned above, then two-step process will be used for polymerization.

Quasi-emulsion solvent diffusion

By using quasi-emulsion solvent diffusion technique porous microsponges can be prepared.In this technique, the first phase is prepared by using eudragit and ethyl alcohol. Then, the active ingredient is added slowly in to the above phase and dissolved. The plasticizers like triethylcitrate (TEC) also added to impart plasticity. The internal phase is poured in the external phase which contains PVA and distilled water with continuous stirring for 2 hours. The product is washed and dried in a hot air oven at 40°C for 12 hr (Çomoğluet al., 2003; Kumariet al., 2016).

w/o/w solvent diffusion

Microsponges can be prepared by double emulsion technique using sodium chloride as a porogenic solution. After that the solution of ethyl cellulose, eudragit and active ingredient in ethanol and dichloromethane is prepared. 1% (w/v) Aqueous solution is prepared using sufficient amount of Span. An aqueous polyvinyl alcohol solution and mucoadhesive polymer is prepared separately and previously prepared w/o emulsion is added to it. This w/o/w emulsion was stirred for 8 hr. The microsponges were obtained by filtration and dried at 60°C in the hot air oven and stored in dessicator till use. A compliation of the advantages and disadvantages of various methodologies used for preparation of microsponges (Table 1).

Drug release mechanism of microsponges

The active moieties are entrapped in porous microspheres. The microsponges consist of an open structure so that active ingredients are free to move through vehicle until equilibrium is attained and vehicle becomes saturated. This results in flow of the drug from the microsponge to the skin.The microsponges are then retained on the surface of the skin and will continue the drug release to the skin and provide a prolonged release for longer period of time. If the drug is freely soluble in the vehicle, the final product will not provide the desired drug release. Therefore, while formulating microsponge, it is important to choose a vehicle which has minimum solubilizing power of the active moities.

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Table 1. A compilation of the advantages and disadvantages of various methodologies used for preparation of microsponges

Method	Advantages	Disadvantages
Liquid--liquid suspension polymerization	Can be suitably modified to one step or two step methods for drug loading	Probable entrapment of unreacted monomers and solvent traces. Non-uniform structure. Requires long time for the reaction of monomers. Requires two-step method for thermosensitive drugs that has low drug loading efficiency
Quasi-emulsion solvent diffusion	No monomer entrapment. Low solvent traces. High drug loading. No exposure of drug to ambient condition. Size of microsponges can be easily controlled by controlling the stirring. Spherical particles	Cannot be used for the loading of water-soluble drugs. Requires long time for the reaction of monomers. Drug should be soluble in a volatile water-soluble solvent
w/o/w emulsion solvent diffusion	Efficient for loading water-insoluble drugs. Can be used to entrap proteins and peptides	Uses water-insoluble surfactants that can be present as residues in the resultant microsponges
Addition of porogen	Highly porous structure with nicely distributed and interconnected pores	May cause disruption in structure
o/o emulsion solvent diffusion	No presence of surfactant traces in microsponges	Requires vigorous washing to remove the traces of organic solvents
Lyophilization	Easy quick reproducible results	May lead to cracking or shrinkage of microparticle
VOAG method	Results in microsponges can be used for targeted drug delivery	Requires reflux conditions
Ultrasound-assisted production	No traces of solvents. Quick and reproducible results	Irregular structure.
Electrohydrodynamic atomization method	Quick reproducible and results	Require cross-linking agents that may be potentially toxic. May lead to the binding of drug molecule to the monomer. Control of size of particle and pores requires expertise.

Microsponges can release the given amount of drug over a period of time. The release is influenced by physicochemical factors like pressure, temperature change and solubility etc.

They are described as follows:

Temperature change

At certain temperature, few entrapped active ingredients become viscous and suddenly get released from microsponges. Increase in temperature of specific region also increases the flow rate and release (Of et al., 2015).

Pressure

When pressure is applied microsponges release the active ingredients at the targeted region (Of et al., 2015).

Solubility

Microsponges are filled with water soluble excipients and they release the drug with water. The release of drug that can be activated by diffusion technique.

pH

pH dependent drug release can be achieved by modifying the coating on the microsponge.

Evaluation of microsponge

Particle size determination

Particle size determination of loaded microsponges can be calculated by optical microscopy. In this sample that can be placed on the slide and mechanical stage. In that mean particle size is calculated by measuring more than 300 particles. For cumulative % drug release of microsponges will be determined by plotting particle size versus time. In the final topical formulation, particles of sizes between 1nm and 25µm are required to be used.

Determination of Production yield and Loading efficiency.

Loading efficiency it can be measured by following equation:

Loading efficiency = Drug Content in Microsponge x 100

Production yield of microsponges can be calculated by the gravimetric method using following equation

Production yield =MMicro/MRM

In that,

Mmicro = Weight of formulated Microsponges.

MRM = Weigh of raw materials (Polymer and active ingredient)

All results can be calculated in the triplicates.

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Characterization of pore structure

In this case, the volume of pore and diameter are very important in controlling the strength and duration of the effect of the drug. Pore diameter also affects the release of drug from the microsponge system through the vehicle in which all ingredients are distributed. By using mercury intrusion porosimetry the pore size of microsponges, percent porosity, the surface area of pore, percent porosity filled, pore diameters, shape and morphology of the pores, void volume, bulk, and apparent density can be determined.

In-vitro release studies

It is done by using dissolution test apparatus USP XXIII with a modified basket having 5μm mesh size. The dissolution rate can be measured at 37°C and 150 rpm. The dissolution media are chosen in order to maintain sink conditions and solubility of active ingredients. Sample aliquots are withdrawn from the dissolution medium and analyzed by a suitable analytical method (UV spectrophotometer) at regular intervals of tim (Naga et al., 2019).

Polymer/ Monomer composition

Various parameters such as spheres size, polymer composition, and drug loading govern the drug release from microspheres. The composition of polymer can also influence the partition coefficient of the trapped active ingredient between the microsponge system and the vehicle, thereby directly affecting the release rate of trapped substance. Drug release of microsponges of the different polymer compositions can be studied by the plotting the graph in-between average % drug release versus time. Polymers exhibiting varying degrees of hydrophobicity or lipophilicity or electrical charges may be prepared to impart flexibility to the release of active ingredients. A variety of probable excipient combinations can be screened for their compatibility with drugs by studying their drug release profile (Barkaiet al., 1990).

Compatibility studies

Infra-red spectroscopy (IR) and thin-layer chromatography (TLC) is conducted to determine the compatibility of drug and excipient. Powder X-ray diffraction (XRD) and Differential scanning calorimetry (DSC) can determine the effect of polymerization or crystallanity of active ingredients. For DSC, approximately 5mg samples are weighed, sealed and heated at 15°C/min in nitrogen atmosphere (Shaha et al., 2010).

Resiliency

Viscoelastic properties (resiliency) of the microsponge system can be tailored to create beadlets which are soft, ion accordance with the requirements of the final formulation. It increases cross-linking and slows down the release rate. Therefore, tests for viscoelastic properties of microsponges are performed and optimized according to prerequisite, considering release a feature of time of interconnection (Shaha et al., 2010).

Physicochemical characterization of microsponges

Scanning electron microscopy

For morphology and surface characteristics, The sample is coated in the gold-palladium at room temperature under an argon atmosphere, and the microsponge surface characteristics can be analysed by scanning electron microscopy(SEM).

Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) is performed for the pure drug, polymer and the drug-polymer physical mixture and microsponge formulations. The samples are incorporated in potassium bromide discs and are evaluated using the FTIR spectrometer. The peaks corresponding to the characteristic bands of the drug must be preserved in the spectra of the microsponges to indicate that no chemical interaction or changes have occurred during the preparation of the formulations.

Powder X-ray diffraction (XRD)

Powder X-ray diffraction (XRD) can be performed for both pure drug, polymer and microsponge formulation to investigate the effect of polymerization on the crystallinity of the drug. The disappearance of the characteristic peaks of the drug in the formulation could indicate that the drug is dispersed at a molecular level in the polymer matrix (Kilmer et al., 2010).

Safety Considerations

- Allergenicity in guinea pigs.
- Eye irritation study performed in rabbits
- Mutagenicity in bacteria
- Oral toxicity study in rats.
- Skin irritation studies in rabbits (Kiliçarslanet al., 2003; Sato et al., 1988).

Limitations

The use of organic solvents poses threats like toxicity and flammability. Traces of residual monomers in the bottom-up approach can be toxic and dangerous to health. But these shortcomings can be overcome by proper quality control measures along with optimization and standardization of procedures e. g, post-manufacture washing (Mandava et al., 2012; Srivastavaet al., 2012).

Applications of microsponges

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This system can be used to increase the effect, safety, and quality of prescription as well as over the counter products. Microsponge drug delivery system can be used in various applications. Microsponges drug delivery is mainly applicable to oral and topical applications. Several patents have been reported using different types excipients due to which microsponges exhibit high loading capacity and sustained release ability. These studies offer the formulator a scope to formulate a wide variety of products. Over the counter (OTC) products that contain microsponge drug delivery system and various sunscreens, specialized rejuvenated products, and moisturizers (Kilmeret al., 2010). Some more application of microsponges give (Table 2). Some examples of microsponge drug delivery with their formulations and uses (Table 3).

Marketed formulations

Microsponges Drug delivery System is ideal for skin and personal care and cosmetic products. They can take up the excess of skin oil while retaining an elegant feel on the surface of the skin. This technology is presently employed in a considerable number of products sold by leading cosmetic and toiletry companies worldwide. These products include oil control lotions, moisturizers, conditioners, deodorants, lipsticks, skin cleansers, powders, makeup and eye shadows which offer various advantages. They are advantageous due to increased chemical and physical stability besides they show greater availability which reduces the skin irritation. The controlled release of the active ingredients and unique tactile qualities are other advantages of this system. Some marketed formulation of microsponges with their advantages (Table 4) with some filed patent related to the microsponges (Table 5).

Recent advances in microsponge drug delivery system

Various advances technology have been made by using different methods or techniques e.g. nanosponges, nanoferrosponges, mucoadhesivemicrosponges, and porous microbeads. β-CD nanosponges were also formulated and can be used for hydrophobic as well as hydrophilic drugs. This nanosponge can be developed by cross-linking the β-CD molecule by reacting the β-CD with diphenyl carbonate. Researchers also observed that incorporating cytotoxic substances in a nanosponge carrier system can increase the potency of the drug, these type of

Table 2. Applications of microspongesystem

Active agents	Applications
Anti-inflammatory e.g. hydrocortisone	Prolonged activity with lessened of skin allergic response and dermatoses.
Anti-dandruff e.g. zinc pyrithione, selenium sulfide	Reduced nasty odour with decreases irritation with increase in safety and efficacy.
Skin depigmenting agents e.g. hydroquinone	Improved stability against oxidation with increase in efficacy and aesthetic application.
Anti-fungals	Sustained release of active ingredients
Anti-acne e.g. Benzoyl peroxide	Reduced skin irritation and maintaining efficacy and sensitivity.
Antipruritics	Extended and improved activity.
Sunscreens	These are long lasting products having high efficacy with enhanced protection againstUv rays, and sunburns, sun related injuries at high concentration and with low irritation and sensitivity.

Table 3. Examples of microsponge drug delivery with their formulations

Microsponge Delivery Systems	Drug	Clinical Use
Gels	TerbinafineHCl	Anti-fungal
	Hydroxyzine HCl	Urticaria and atopic dermatitis
	Acyclovir	Viral infections
	Fluconazole	Inflammation
	Benzoyl peroxide	Anti-Acne Treatment
Lotions	Benzoyl peroxide	Anti-Acne Treatment
Creams	Hydroquinone and Retinol	Melanoma
Tablets	Indomethacin	Inflammation
	Paracetamol	Anti-pyretic
	Chlorpheniramine maleate	Hay Fever
	Ketoprofen	Musculoskeletal pain
	Paracetamol	Colon targeting
Implants	Poly (DL-lactic-co-glycolic acid)	Skin tissue engineering
Grafts	Poly (lactic-co glycolic acid)	Cardiovascular surgery
Injection	Basic fibroblast growth facto	Growth factor

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Table 4. Marketed formulations of microsponges

Product name	Manufacturer	Advantages
Carac Cream	Dermik Laboratories, Inc. Berwyn , PA 19312 USA	Carac Cream contains 0.5% fluorouracil; it includes 0.35% incorporated in a porous microsphere consisted of methyl methacrylate / glycol dimethacrylate cross-polymer and dimethicone. Carac is a once-a-day topical application . For the treatment of actinic keratosis caused by over- exposure to the sun.
Retin-A-Micro	Ortho-McNeil Pharmaceutical, Inc.	Retin-A-Micro contains 0.1% and 0.04% tretinoin entrapped into a porous microsphere consisted of methyl methacrylatedimethacrylate cross-polymer to enable inclusion of the active ingredient, tretinoin, in an aqueous gel. Used for the topical treatment of acne vulgaris.
Salicylic Peel 20 & 30	Biophora	Salicylic acid 20% has been used in to it.Microspongesystem used for stimulat the skin for for faster results. Itimprove pigmentation, fine lines and acne. Salicylic acid passes easily through the pores.
Line Eliminator Dual Retinol Facial Treatment.	Avon	Retinol (Vitamin A) in MicrospongesDrug Delivery Systeem, for wrinkle-fighting action it release by two ways like immediate and timely release of drug. It clearly reduses appearance of lines and wrinkles.
Micro Peel Plus /Acne Peel	Biomedic	It stimulates the cell turnover so the application of salicylic acid in the form of microcrystals,These microcrystals target the specific areas of the skin. It is the chemical peels releases in to the skin of all dead cells while doing no damage to the skin.
Retinol cream, Retinol 15 Night cream	Biomedic, Sothys	Night cream. Microsponge technology it conatains pure retinol, Vitamin A. It diminishment of fine lines and wrinkles,
Lactrex™ Moisturizing Cream	SDR Pharmaceuticals, Inc., Andover , NJ , U.S.A. 07821	Natural humectant is used for soften and help to moisturizing the dryskin, cracked skin. It also contains 12% lactic acid as a neutral ammonium salt, ammonium lactate,water and glycerine.
Oil free matte block spf20	Dermalogica	Oil-free sunscreen protect the skin from damaging UV-rays while controlling the oil production and givesyou a healthy matte finish. That can be formulated with microsponge technology, Oil free matte block absorbs oil and prevents the shine without any powder esidue.
Sportscream RS and XS	Embil Pharmaceutical Co. Ltd.	Topicalprepareation It gives analgesic-anti-inflammatory and counterirritant actives for the management of musculoskeletal conditions.
Oil Control Lotion	Fountain Cosmetics	Microsponges that can absorb the oil from surface of skin, Eliminatethe shine for hours with this feature-weight lotion, formulated with oil-absorbing Microsponge technology. It can be mainly use for the Acne-Prone, oily skin conditions.s

carriers can be used mainly for the targeting the cancerous cells (Hu et al., 2007). Nanosponge, a novel approach constitutes the self- performing carriers having better penetration to the targeted site due to the external magnetic triggers which enforce the carriers to penetrate to the deeper tissues. Thereafter, the removal of magnetic material from the particles is effected leaving a porous system (Cavalli et al., 2006). The improved characteristics of porous microspheres, led to the development of a process to produce the porous microbeads. This method (High internal phase emulsion, HIPE) consisted of the monomer containing continuous oil phase, a cross-linking agent and aqueous internal phase (Çomoğlu et al., 2007). They also observed increased stability of RNA and the relatively effective encapsulation process of siRNA. This approach may lead to novel therapeutic routes for siRNA delivery (Lee et al., 2012).

Future prospects

Microsponge drug delivery system holds a promising opportunity in various pharmaceutical applications and industry in the coming future as it has unique properties like enhanced the product performance and elegancy, extended the release of active moieties, improved drug release

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Table 5. Patents Filed Related to Microsponges

Patent no	Inventors	Publication Date
US4690825	Won, Richard	1987
US4863856	Dean RC Jr et al.	1989
US5292512	Schaefer et al	1989
US5135740	Katz et al.	1992
US5679374	Fanchon; Chantal et al	1994
US5316774	Eury, Robert P et al.	1994
US5725869	Lo; Ray J. R.	1996
US6395300	Straub et al.	1999
US6211250	Tomlinson et al	2001
US20030232091	Shefer et al.	2002
US20040247632	Cattaneo, Maurizio	2004
US20050271702	Wright, Steven G et al.	2005
WO2008097429A1	Franklin Sadler Love	2007

profile, reduced irritation, improved physical, chemical, and thermal stability which makes it flexible to develop novel formulations. The real challenge in the future is the development of the delivery system for oral peptide delivery by changing ratios of polymers. The use of bioerodible and biodegradable polymers for drug delivery enables it for the safe delivery of the active material. These porous systems have also been studied for drug delivery through a pulmonary route, which shows that these systems can show effective drug release even in the scarce of the dissolution fluid. Therefore, colon is an effective site for targetted drug release. Development of carrriers for alternative drug administration routes like parenteral and pulmonary route is necsessary. These particles can also be used as cell culture media and thus can also be employed for stem cell culture and cellular regeneration in the body. These carrier systems have also found their application in cosmetics due to their elegance,. These developments enabled researchers to utilize them for various purposes. These novelties in the formulation also a new way for drug delivery (Srivastava et al., 2012)

Conclusion

With the demand for innovative and highly efficient Pharmaceutical as well as Cosmetic products, the market holds considerable potential for Microsponge technology and the versatility they offer. Since the researchers have found the new and creative way to deliver actives moieties, they can realize that the full capability of these materials providing safety and stability. It also reduces side effects of the active moieties, enhances multi-functionality and also increases active ingredient compatibility with the excipients. Microsponge delivery system would be a winning and innovative strategy for future, in the Pharmaceutical and Cosmetic industry. Microsponges have a distinct advantage over the conventional topical dosage forms for the treatment of topical diseases; it is a new strategy or one of a kind of technology for the controlled release of agents. It is advantageous over other products by because it is non-mutagenic, non-toxic & non-irritant. Thus the microsponge drug delivery system has got a lot of potential and is an emerging field which is essential to be explored for research in future.

Authors contribution

All the authors have contributed to the preparation and editing of this systematic review article.

Conflict of interest

The authors declare that they have no conflict of interest.

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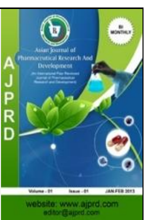

Review Article

A Review on Medicinal Plants of Natural Origin for Treatment of Polycystic Ovarian Syndrome (PCOS)

Shivani Andhalkar *[1], Vitthal Chaware [2], Vivek Redasani [3]

[1]Department of Pharmacology, YSPM's Yashoda Technical Campus Wadhe, Satara, India.
[2]Head of Department (Pharmacology), YSPM's Yashoda Technical Campus, Wadhe, Satara, India.
[3]Principal, YSPM's Yashoda Technical Campus, Wadhe, Satara, India.

A B S T R A C T

Polycystic ovarian syndrome (PCOS) related infertility is a global problem that is spreading at an alarming rate. Chronic anovulation, polycystic ovaries, and hyperandrogenism, as well as abnormal menstrual cycles, hirsutism, acne, and infertility, are all symptoms of this condition. PCOS is linked to insulin resistance and elevated levels of male hormones (androgens). Among other things, an inactive lifestyle, a lack of exercise, dietary changes, and stress are all contributing factors. Curcuma longa, Aloe barbadensis**,** Mentha piperita, Allium fistulosum Cinnamonum zeylanicum, and other plants have been shown to be effective in the treatment of PCOS. The aim of this review is to summarise the most effective medicinal plants that are used in the treatment or prevention of PCOS. Special emphasis is placed on the role of insulin resistance and the possible utility of insulin sensitizers in the treatment of PCOS.

Keywords- Polycystic ovarian syndrome (PCOS), Screening methods of pcos, Pathophysiology of pcos.

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***Address for Correspondence:**

Andhalkar Shivani, Department of Pharmacology, YSPM's Yashoda Technical Campus Wadhe, Satara, India

INTRODUCTION

Leventhal and Stein in 1935 first defined a disorder, which would ultimately become known as polycystic ovary (or ovarian) syndrome (PCOS)[1]. Polycystic ovary syndrome (PCOS), a unitization of symptoms, which affects women of child-bearing age is assumptive in epidemic proportions. A resultant of imbalance in proportion of female sex hormones, results in cysts within antral follicles of ovaries. Once multiple cysts are formed in the ovarian follicles because of the hormonal imbalance, it is characterized as PCOS. Anovulation and absence of menstrual cycle prevents fertilization, and conception in women, thus pregnancy becomes troublesome[2]. PCOS affects 6–10% of women throughout the globe. According to 1990 NIH criteria 7–12 and even more individuals. According to the broader Rotterdam criteria, which makes it one of the most common human disorders and the single most common endocrinopathy in women of reproductive age.[3] The oxidative stress (OS), that will increases in inflammation, which also been reported as possible cause of PCOS[4]. Women with PCOS has several risk factors which are associated with the development of uterine cancer including fatness, hyperinsulinemia, diabetes mellitus and abnormal uterine bleeding.[5] The frequency of depression and anxiety is higher in women with PCOS than in the general population. Mood disorders are capable of impairing quality of life, which are well-known in young adult women, concerned with fertility, and in women of all ages with respect to obesity, and clinical manifestations of excessive androgen.[8]

RISK FACTORS[6]

- Obesity
- Family history of Infertility
- Family history of PCOS
- Family history of diabetes
- Fast food diet habits
- Lack of physical exercise

PATHOPHYSIOLOGY OF PCOS[7]

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The pituitary gonadotropin is fundamental to reproductive function-its production and secretion of FSH and LH is directly stimulated by hypothalamic GnRH and it is also influenced by integrated feedback mechanisms. The initial stimulus for follicular development and also granulosa cell conversion of androgens to oestrogens by stimulating the aromatase enzymes is provided by FSH. Luteinizing Hormone(LH) characteristically known for its role in the luteal phase by promoting secretion of progesterone, also it has a vital role in the follicular phase, for inducing thecal androgen production. Women with PCOS often secrete more LH and this might result in higher theca cell androgen secretion. to maintain gonadotropin secretion pulsatile GnRH stimulation is required, but the continuous exposure of the pituitary to GnRH causes desensitisation and a suppression of gonadotropin secretion. Due to Changes in the pulsatility of GnRH alter the ratio of secretion of the two pituitary gonadotropins throughout the menstrual cycle.[7]Excessive androgen in PCOS is related with increase in abdominal fat leads to dyslipidemia and hyperinsulinemia. Thus, hyperinsulinemia reduces hepatic sex hormone-binding globulin(SHBG) to increase circulating bioactive testosterone levels.[8]

Screening Methods of PCOS

Androgen Induced PCOS Model[9]:

Hyperandrogenism is the most common symptom of PCOS. One of the etiologic hypotheses for PCOS is that early life exposure to excessive androgens leads to PCOS later in life. Increased levels of circulating androgens in the rodent affected ovarian follicular maturation and cyst development, according to a study published more than 30 years ago. Several androgens, including dehydroepiandrosterone (DHEA), testosterone propionate (TP), and 5a-dihydrotestoterone, have been used to induce an acute PCOS condition in rats through regular injection or subcutaneous implants (DHT). However, there is still some inconsistency in the reporting of endocrine hormones and ovarian histology in different models. Furthermore, some studies did not look at cardiometabolic parameters or the effects of daily androgen injections and/or treatment on physiologic indices like body weight, stress indicators, or food intake. The pathological induction of PCOS in these rodent models is transient and dependent on androgen treatment. As a result, natural reproductive/ovarian cycling happens again after androgen administration is stopped.

DHEA Induced PCOS[9]:

The first androgen to increase in the female peripubertal cycle is dehydroepiandrosterone. Nearly half of follicular synthesised T can be obtained from circulating DHEA, and 25% of PCOS patients have higher-than-normal circulating DHEA levels. Roy et al. were the first to use dehydroepiandrosterone to induce PCOS in rats. DHEA (6 mg/100 g body weight, dissolved in 0.2 mL sesame oil) is injected daily for up to 20–27 days into prepubertal rats, typically aged 22 days. Rats become acyclic and anovulatory after treatment

Ovarian Morphology: Multiple follicular cysts varying in size from 0.45 to 2.2 mm in diameter, as well as degeneration of granulosa cell layers, grow in dehydroepiandrosterone-induced rats. The ovarian tunica capsule is not thickened, and the ovarian weight of DHEA-treated rats is substantially increased.

***Endocrine hormone profile*:** DHEA-induced rats have significantly higher serum DHEA, T, E2, FSH, LH, and PRL concentrations than control rats, while no changes in plasma FSH and LH concentrations have been identified by other groups. Fasting serum glucose and insulin concentrations were higher in DHEA-induced rats, indicating cardiometabolic abnormalities.

Early DHEA-related hyperandrogenemia, anovulation, cystic ovaries, and the production of insulin glucose metabolism abnormalities can all be detected using the DHEA-induced model.

TP- *Summary*: Induced PCOS Model[10]:

Testosterone propionate (TP) can cause hyperandrogenemia in rats when given prenatally or postnatally. Furthermore, prenatal T exposure during the crucial time of foetal development has been linked to reproductive system developmental and morphological abnormalities. Pregnant rats were given a single dose injection of T on gestational day 20 or T propionate (TP) from days 16 to 19 (3 mg T daily) of pregnancy for prenatal administration. Rats were given TP at a dose of 1.25 mg/100 g body weight at 5 days of age, or daily injections of 1 mg/100 g body weight from 21 to 56 days of age.

Estrous cyclicity: T prenatally treated rats had longer and more irregular estrous periods. Estrous cyclicity was disrupted and diestrus phase was persistent in postnatally treated rats.

Ovarian morphology: In the ovaries of rats treated prenatally with T, the number of preantral and antral follicles increased, whereas the number of pre-ovulatory follicles and corpus luteum (CL) cells decreased, as opposed to control rats. In prenatal T-treated rats, cystic follicles were also discovered. Rats given T postnatally, on the other hand, had massive cystic or atretic follicles and luteinization of theca cells in the ovaries. When postnatal T treated rats were fed a high fat diet, their body weight increased while their fasting glucose levels remained unchanged.

Summary: The ovary of rats treated with postnatal T showed morphological changes that mirrored the human PCOS phenotype. Prenatal T therapy, on the other hand, increased the number of preantral and antral follicles in rats, despite the fact that cystic follicles and ovary weight were unchanged in this model, and the reported changes did not match the ovarian morphology of people with PCOS. Both prenatal and postnatal T therapy increased serum T levels. Prenatal T administration had no effect on serum E2 and P4 levels, while continuous postnatal T treatment increased E2 levels, likely due to T conversion. An increased number of kisspeptin-positive cells in the ARC of

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prenatal T-treated ewes may be linked to defects in GnRH/LH secretion feedback control 11; however, one drawback of this study is that LH levels and ovarian morphology were not examined..

DHT induced PCOS models[10]:

Since DHT is not converted to E2 by aromatase, the PCOS phenotype in DHT-treated animals can be studied without taking into account the effects of oestrogen derived from androgens.

Prenatal DHT treated models:

Mice were injected with 250 g of DHT on days 16, 17, and 18 of gestation 28 to generate prenatal DHT-treated animals, while rats were given 3 mg of DHT daily from gestational day 16 to 19. The offspring were used as PCOS models that had been prenatally treated with DHT.

Estrous cyclicity: Prenatally administered DHT caused irregular cycles in rats and mice. The mice spent more days in diestrus and fewer days in proestrus than controls, resulting in fewer litters being produced every three months.

Ovarian morphology: Prenatal DHT treatment resulted in fewer normal large, antral, preovulatory follicles and CLs, as well as more atretic cyst like follicles. In prenatal DHT treated mice, CL and antral follicle wall areas were reduced, but the number of atretic cyst like follicles and the thickness of the antral follicle theca cell layer increased.

Neuropeptides in the hypothalamus: The number of kisspeptin and NKB positive cells in the ARC of the hypothalamus increased significantly in prenatal DHT treated rats, whereas the number of kisspeptin positive cells in the AVPV did not differ from that of control animals in diestrus. The input of aminobutyric acid (GABA) to GnRH-expressing neurons was increased in mice given DHT prenatally, according to a recent study.

Metabolic features and adiposity: Prenatal DHT-treated rats and mice had body weights that were close to control animals. Prenatal DHT therapy, on the other hand, increased adipocyte region in parametrial fat and the degree compared to the control group.

DHT prenatally treated rats and mice had abnormal estrous cycles and ovarian morphology similar to PCO. In prenatal ***Summary:*** DHT-treated rodents, increased LH levels were observed, along with an up regulation of kisspeptin in the ARC. There was no discernible difference in body weight, on the other side. This phenotype is similar to PCOS, which is marked by normal body weight and increased LH secretion.

Letrozole-Induced (Aromatase Inhibitor) Rodent Model of PCOS[11] :

Abnormal follicular development and polycystic ovary may result from intraovarian androgen excess caused by circulating hyperandrogenemia or abnormal steroidogenesis. P450 aromatase, which was expressed in the placenta, ovary, and testis as well as a wide variety of human tissues, converted testosterone and androstenedione into estradiol and estrone, respectively; a decrease in the enzyme's activity could result in increased ovarian androgen production and the development of PCOS. Aromatase is the key enzyme that converts T and androstenedione into E2 and estrone, respectively. It is widely expressed in human tissues, such as placenta, ovary, and testis. Reduced aromatase activity in the ovary is one of the pathophysiologic hypotheses of PCOS development. Letrozole is a nonsteroidal aromatase inhibitor that reduces conversion of androgens to estrogens in the ovary, resulting in increased T and decreased E2 production. Excess T in the ovaries is likely to cause polycystic ovaries directly in Letrozole-treated rats. The reduction in estrogen weakens the negative feedback on LH production in the pituitary, resulting in increased LH levels, which further stimulates theca cells to secrete T. Typically, 6-week-old female rats (puberty) are administered Letrozole orally at doses of 0.1, 0.5, and 1.0 mg/kg daily for 21 days, after which they become acyclic, with histological and biochemical features of human PCOS.

Estrous cyclicity: Regular vaginal smear examinations were used to monitor estrus cycles. In the analysis, only animals with two consecutive standard 4-day periods were used. The rats and mice treated with letrozole were fully acyclic. This rat model's vaginal smears showed an excess of leukocytes, the diestrus phase's predominant cell type.

Ovarian morphology: Ovaries from control group exhibited follicles in various stages of development including secondary follicles, graffian follicles, and fresh corpora lutea. In study groups, letrozole inhibited growth of follicles in a dose-dependent manner. Small follicles could be observed in early development, in addition to follicles showing evidence of atresia, and many large cysts with virtually no granulosa cell layer or large cystic follicles with scant granulosa cells. Ovaries from the sample groups had a higher rate of subcapsular ovarian cysts and capsular thickening than the control group. together with incomplete luteinization and a dose-dependent decrease in the amount of corpora lutea In some of the research classes, there was also evidence of theca cell hyperplasia..

Summary: Acyclicity, cystic ovarian morphology, elevated serum LH levels, and higher Kiss1 mRNA expression in the posterior hypothalamus are all observed in letrozole-induced PCOS model rats compared to control rats. This model accurately reproduces the metabolic characteristics of human PCOS, including a PCO-like morphology and elevated serum LH levels, and is thus suitable for studying human PCOS. Increased KNDy neuron activity was linked to a reduction in the negative feedback effect of sex steroid hormones, as evidenced by increased Kiss1 mRNA and serum LH levels.

Medicinal plants of natural origin-

Curcuma Longa (Turmeric) ***[12]:***Curcumin is a water-insoluble polyphenolic curcuminoid derivative found in the rhizomes of Curcuma longa, an Indian spice (turmeric). Turmeric is widely used in Asian cuisine as a food additive

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and colouring agent, as well as in Indian herbal medicine. Curcumin makes up around 2–8% of all turmeric preparations. Curcumin has been shown to have a wide range of biological effects like Anti-inflammatory, antioxidant, hypoglycaemic, and antihyperlipidemic properties The study used virgin, cyclic, adult female Wistar Albino rats weighing 160–200 g. Once everyday for 21 days, followed by treatment with curcumin. Letrozole treatment resulted in abnormalities in the serum sex steroid profile, lipid profile, glucose, and glycosylated haemoglobin levels and antioxidant activity has been depleted. Whereas Curcumin was able to exert its calming effect by returning all parameters to normal and causing cysts in the ovaries to vanish. Curcumin, like Clomiphene citrate, has a number of beneficial effects in the treatment of PCOS.

Aloe barbadensis (Aloe)[13]:Aloe barbadensis Mill. (Liliaceae) is a well-known plant with such properties. Polyphenols, sterols, flavanoids, and other nutrients were analysed qualitatively and quantitatively for polyphenols, sterols, flavanoids, and other nutrients in the Aloe vera gel formulation. To induce PCOS, five-month-old Charles Foster female rats were orally fed letrozole, a non-steroidal aromatase inhibitor. The rats were then given the Aloe vera gel formulation orally.AVG treatment of PCO rats resulted in a reduction in ovary atretic cysts as compared to PCOS controls, according to histological review. By restoring ovarian steroid status and modifying main steroidogenic behaviour, aloe vera gel formulation protects against the PCOS phenotype.

Glycyrrhiza glabra (liquorice)[14]: Traditional medicine has used liquorice (Glycyrrhiza glabra of the Leguminosae family) to treat a variety of ailments. Antifungal, antiviral, antibacterial, and antihyperglycemic properties are all present in it. The most bioactive compound in liquorice is glycyrrhizic acid. Phytoestrogens found in liquorice include liquiritigenin, liquiritin, isoliquiritin, isoliquiritigenin, glabidin, and glabrene. The effects of two natural compounds derived from liquorice root on vascular tissues in vitro and in vivo were reported: glabridin, the main glabrene, and isoflavane, an isoflavene, both demonstrated estrogen-like activities. One of the bioactive compounds responsible for weight loss may be liquiritigenin, a selective oestrogen receptor ligand. Some molecules, such as glabrene and glabridin, have been shown to reduce weight in vivo. It has also been documented that treating hirsute women with a combination of spironolactone and liquorice may help with PCOS by reducing the volume depletion caused by spiranolactone and possibly increasing its anti-androgenic activity.

Mentha piperita(Peppermint) [15]:Peppermint (Mentha piperita L.) is a member of the Labiatae family that originated in the Mediterranean region and is now widely cultivated all over the world. Antioxidant, antitumor, antiallergenic, anti-inflammatory, antiviral, antibacterial, and antifungal properties are all present in peppermint. It also has anti-androgenic properties, lowering the level of free testosterone in the blood after three weeks of treatment with letrozole and peppermint. Females with PCOS had significant changes in serum testosterone, oestrogen, LH, and FSH function. Ovarian cysts with a reduced granulosa layer, atretic follicles, and a small number of corpora lutea were found in the PCOS community. Peppermint was found to have a strong potential as an alternative therapy in the treatment of PCOS, as shown by necrosis in stromal mesenchymal cells, hyperplasia of luminal epithelial cells, and necrosis in stromal mesenchymal cells.

Allium fistulosum (Onion)[16]: In Asian countries, the Welsh onion (Allium fistulosum) is well-known for its use in food and traditional medicine. For treatment, administered AF extract to letrozole-treated rats for 2 weeks. In terms of serum hormonal levels, the LH/FSH ratio and serum oestrogen levels were positively affected by AF extract therapy. FSH and LH are necessary for ovulation, and PCOS patients often have a two- to three-fold increased LH/FSH ratio, which is enough to cause ovulation disruption. The findings suggest that AF extract normalises follicular growth and ovarian cysts. In the letrozole-induced PCOS rat model, the steroid hormone-related receptors demonstrated restoration of m-RNA expression after treatment with AF extract A. fistulosum extract treatment relieved hormonal imbalance and altered ovarian function.

Linum usittassimum (Flaxseed)[17]: Flaxseed is made from Linum usittassimum (Linaceae), an omega-3 fatty acid-rich food that is also one of the best sources of dietary lignin. ALA, lignans (secoisolariciresinol diglycoside-SDG), and soluble flaxseed fibre mucilage (d-Xylose, L-Galactose, L-Rhamnose, d-galacturonic acid) are all biologically active compounds with major health benefits. Flaxseed or isolated lignan has been shown in studies to lower androgen levels while also normalising lipid levels. Lignans seem to minimise excess testosterone, which is a crucial factor in the development of PCOS. Flaxseed supplementation can help women with PCOS control androgen levels, according to a case study. The study found a substantial reduction in androgen levels. There was also a decrease in hirsutism. Flaxseed can have a significant effect on testosterone levels, as well as symptoms associated with hyperandrogenism, such as hirsutism, according to the findings. Another research looked at the impact of flax seeds on ovarian morphology in PCOS patients, finding that flax seed supplementation decreased ovarian volume, increased the amount of follicles in the ovaries, and improved menstrual cycle duration. However, hirsutism, blood sugar levels, or body weight did not improve as a result of the research.

Panax ginseng (Ginseng)[18]: Herbal medication is made from the roots of Panax ginseng (Araliaceae). It has anti-aging properties and is used as a tonic. Ginseng saponins are ginseng's active ingredient. Rb1, Rb2, Rc, Rd, Re, Ro, Ra, and minor ginsenosides make up these ginsenosides. It can be used as a dietary supplement. Estradiol valerate induced polycystic ovary in rats. The ovarian morphology was examined in this analysis. The ginseng-containing formulation is known as Kampo preparations**.** It is formulation significantly decreases the plasma LH levels

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and thereby it is effective in improving endocrine condition in the treatment of disturbances of ovulation in patients with PCOS.

Tribulus terrestris (Puncture vine)[19]: Puncture vine or Devil's eyelashes, Tribulus terrestris (Zygophyllaceae), plays an important role in traditional medicine. The herb Tribulus terrestris has been shown to help with polycystic ovarian syndrome. Tribulus terrestris extract was found to be successful in improving ovulation in rats with polycystic ovaries induced with estradiol valerate in a study. The extract treatment improved ovarian follicular development and normalised estrous cyclicity and steroidal hormone levels. Many herbalists believe that tribulus is an excellent overall ovarian stimulant and female fertility tonic for women with polycystic ovary syndrome.

Gymnema sylvestre (Gymnema)[20]: Gymnema sylvestre (Asclepiadaceae) is an Ayurvedic herb that has been used for thousands of years. It has a wide range of pharmacological effects, including anti-diabetic, hypoglycemic, and lipid-lowering properties. Saponins, especially gymnemic acids, are the active constituents in gymnema. Gymnema has been shown to have hypoglycemic properties in diabetic animal models. It keeps blood glucose levels in check. Metformin therapy is a convenient way to treat PCOS. Gymnema can thus be used to treat the root cause of insulin resistance. Gymnema is a good choice for PCOS because of its insulin-modulating properties and the added advantage of lowering the high triglycerides that come with the condition.

Punica granatum (Pomegranate)[21]: Pomegranate (Punica granatum of the Punicaceae family) is a fruit with a wide range of medicinal properties. Folic acid, vitamins (B2, C, B1), carbohydrates, pantothenic acid, and organic acids are all contained in the fruit. Unsaturated and saturated fatty acids are said to be present in the crop. In adult female rats, the effect of pomegranate extract in the control or management of PCOS was studied using a control and a PCOS community. The levels of free testosterone, serum oestrogen, and andrestandoin hormone were measured in the experimental community. Pomegranate extract seems to have a protective impact on polycystic ovarian syndrome hormonal imbalances, according to the report. The extract's phenolic compounds and phytosterols have been shown to help alleviate PCOS complications. Consumption of the extract, according to the report, decreases the complications associated with PCOS.

Symplocos racemosa (Lodh Tree)[22]: Symplocos racemosa Roxb, a member of the Symplocaceae family, is a common Ayurvedic remedy for female problems. It's also known as Lodhra, and it's used as a single medication or in multi-component formulations and preparations in Indian medicine. In a Letrozole-induced female rat model, the anti-androgenic properties of S. racemosa were investigated in the treatment of PCOS. Treatment with Symplocos racemosa resulted in substantial improvements in oestrogen, testosterone, progesterone, and ovarian tissue levels. It improves fertility and prevents ovarian cell dysfunction in PCOS patients.

Cinnamomum zeylanicum (Cinnamon)[23]: Cinnamon (Cinnamomum zeylanicum of the Lauraceae family) is an insulin potentiator. Insulin-stimulated glucose uptake and glycogen synthesis are controlled by this compound. Fasting and oral glucose tolerance test values were assessed in fifteen women with PCOS in a pilot study. In women with PCOS, the cinnamon extract increased insulin sensitivity. Cinnamon extract contains polyphenols and procyanidins, which potentiate the insulin signalling pathway, resulting in a hypoglycemic impact. Cinnamon's function as an adjunctive therapy in the treatment of PCOS was identified in this research. Cinnamon's impact on menstrual cyclicity and metabolic dysfunction in women with PCOS was studied in another research. It was a 45-woman randomised controlled trial. Oral cinnamon supplements were given. Menstrual cyclicity, luteal phase, and progesterone levels were all tracked. Cinnamon supplementation increased menstrual cyclicity and was shown to be beneficial in the treatment of polycystic ovary syndrome.

Vitex Negundo(Chaste Tree)[24]:Vitex negundo is a plant belonging to the (Linn) Verbenaceae family, genus Vitex, and species negundo. It's the five-leaved chaste flower, also known as monk's pepper. It has been documented to have anti-inflammatory, analgesic, antioxidant, antifungal, antiviral, and anti-inflammatory properties, as well as being used in gynaecological disorders. It also has anti-androgenic and estrogenic properties (linoleic acid-like estrogenic compounds). For the induction of PCOS, letrozole was given orally (p.o) for a duration of 21 days. The rats were then given extract of vitex negundo, which has positive effects on the ovary as well as effects on glucose tolerance, estrous cycle irregularities, LH: FSH ratio, steroidogenic enzymes, and cardiovascular parameters. It was able to successfully treat the rats with extract, which caused abnormalities in serum sex steroid profile, lipid profile, glucose, and estrous cycle. This may be attributed to the extract's phyto-components.

CONCLUSION:

The most common cause of menstrual irregularities and hyperandrogenism is polycystic ovary syndrome (PCOS). It is the most common cause of female infertility. Several risk factors for PCOS have been studied, including glucose intolerances, obesity, and dyslipidemia. Many treatments are currently available, but they are associated with moderate to serious side effects, and their high cost has led to a search for plant-based remedies to treat PCOS. In this study, summarize some of the most important medicinal plants for treating PCOS and helps with PCOD symptom relief and management. Hyperandrogenism, insulin sensitivity, fertility, and menstrual cyclicity are all aided by these plants.

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REVIEW ARTICLE

Efficiency of AUTODOCK: *Insilico* study of Pharmaceutical Drug Molecules

Uday M. Satpute*, Sachin H. Rohane
Department of Pharmacy, Yashoda Technical Campus, Satara - 415015, India.
*Corresponding Author E-mail: **udaysatpute650@gmail.com**

ABSTRACT:

In current situation lots of Molecular Docking Software are available in the market for *Insilico* study of pharmaceutical drug molecules, so that we have to choose most appropriate software. During literature survey it was found that AUTODOCK software was efficiently guided to as author's regarding mainly pharmacological activities such as Antidiabetic activity, Antimalarial activity, Antivirus activity, Anticancer activity, Anti mycobacterial tuberculosis activity, Antioxidant activity, Etc. After review it was analyse that the AUTODOCK and its Tools are more efficient to determine the synthesis techniques, spectral analysis, docking simulation, photochemical activities, therapeutic effects, toxicological studies.

KEYWORDS: Molecular docking, Drug discovery, Auto dock software.

INTRODUCTION:

In the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using, for example, scoring functionsThe associations between biologically relevant molecules such as proteins, peptides, nucleic acids, carbohydrates, and lipids play a central role in signal transduction. Furthermore, the relative orientation of the two interacting partners may affect the type of signal produced (e.g., agonism vs antagonism). Therefore, docking is useful for predicting both the strength and type of signal produced.

Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site. Characterisation of the binding behaviour plays an important role in rational design of drugs as well as to elucidate fundamental biochemical processes [1-3].

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REVIEW OF LITERATURE:

Sukanta Kumar Pradhanand et al used the Autodock 4 softwear for conducting several studies and identifing several target sites which influence drug-resistant to M. tb strains. In this case, there is the interaction between the protein Arabinosyl transferase C and two existing drugs (Ethambutol and Isoniazid) and by calculating the binding affinity and mode of binding. Ethambutol formed the five modified molecules (Emb 1, Emb 2, Emb 3, Emb 4 and Emb 5). The Emb 1 and Emb 3 having binding affinity- 5.77Kcal/mol and -5.13Kcal/mol respectively that are potential inhibitors of Arabinosyl trasferase C in mycobacterium tuberculosis [4].

Mohammed Al bratty used the Autodock Vina softwear for studying the extent and types of binding interaction present in between HAS and Anti Hypertensive drug like telmisartan (TLM). HAS is responsible for binding and transportation of many exogeneous and endogeneous ligand including drug like telmisartan in binding interaction, the TLM significantly interacts with binding site-1 of HAS by forming strong Hydrogen with Glu292 and Lys195 residues that affects concentration of TLM at site of action and also affects on therapeutic effect [5].

Idhayadhulla Akabar and et al used Auto Dock Vina softwear for the molecular interaction study between target protein and ligands. This study more exposed the all inhibitor acquired the negative dock energy against

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the target protein. In molecular docking investigation it was found that the natural coumarin analogue toddacoumaquinone shows the inhibitory activity with binding energy -7.8Kcal than other compound against main protease of SAR coronavirusin intricate with a-ketomide [6].

Ran Yu and et al used the AutoDockVina softwear in the current spread of noval coronavirus (SAR-CoV-2) situation to discover the Anti Viral drug. This softwear helps to screening the potential drug by molecular docking with structural protein and non-structuralprotein site of new coronavirus. The Ribavirin, remdesivir, chloroquine and luteolin are also studied, the luteoline is bind with high affinity to same site of the main protease of SARS – CoV-2 [7].

Fareeda Athar and et al used the AutoDock. 4.2. for molecular docking studies performed that all derivatives along with the standard inhibitor STX-0119 showed that binding energy released in direct binding with the SH2 domain of STAT3 was the highest for compound 5e (-9.91kcal/mol). In virtual screening, compound 5e was found to exhibit optimum competency in inhibiting STAT3 activity. Compound 5e decreased the activation of STAT3 as observed with Western blot. The compound 5e was identified as a potent antioxidant agent and STAT3 inhibitor and effective agent for cancer treatment [8].

Akinwunmi O. Adeoye and et al used AutoDock softer for Molecular docking and virtual screening to understand the mechanism of ligand binding and to identify potent calcium transporter inhibitors: This study also deals with the evaluation of inhibitory activity of secondary metabolites of ethylacetate partitioned-fraction of Adansoniadigitata stem bark extract on malaria-associated protein using in silico docking studies. Digitata stem bark extract were examined for their antiplasmodial activity. Digitata shows the binding energy ranging between -6.5kcal/mol and -7.1kcal/mol. Among the two chemical constituents, apigenin has the highest docking score along with the highest number of hydrogen bonds formed when compared to quercetin. The analysis results suggest that apigenin and quercetinare acts as an anti-malaria agent [9].

Mohammad Jakir Hosen and et al used the AutoDock Vina softwear for the screening of the drugs against RdRp of SARS-CoV-2. It has been found that RNA dependent RNA polymerase (RdRp) plays a crucial role in SARS-CoV-2 replication, and thus could be a potential drug target. This study revealed that Rifabutin, Rifapentine, Fidaxomicin, 7-methyl-guanosine-5′-triphosphate-5′-guanosine and Ivermectin have a potential inhibitory interaction with RdRp of SARS-CoV-2 and could be effective drugs for COVID-19 [10].

PrashamsaKoirala, Su HuiSeongand et al used the AutoDock 4.2 softwear for determined the molecular interaction of BACE1 with isolated terpenoids. The AutoDock 4.2 programme revealed that hydroxyl group of lupeol formed two hydrogen bonds with the ASP32 (catalytic aspartic residue) and SER35 residues of BACE1 with the binding energy of (−8.2kcal/mol), while the ketone group of lupenone did not form any hydrogen bonds with BACE1 giving evidence for less binding affinity. It predicted that the dependence of the inhibitory activity in the presence of hydroxyl group which has provided a new basis for BACE1 blockade [11].

A. Lakshmana Rao and et al used the AutoDock 4.2.6softwear. The docking procedure was applied on a set of designed ligands within the region of 2PRG active site using AutoDock 4.2.6 software. Based on the validations and hydrogen bond interactions of various substituents, they were considered for the evaluation. It was done to understand the kind of interactions that occurred between various substituted thiazolidine-2, 4-diones with 2PRG binding site region [12].

Lakshmana Rao ATMAKUR and et al used AutoDock softwear for In vitro anti-inflammatory activity was checked by human red blood cell (HRBC) membrane stabilization and protein denaturation. Using AutoDock, molecular docking studies were carried out to find out the best fit ligands. In molecular docking studies, compounds 4h and 4n exhibited higher binding affinity at PPARγ receptor protein and compound 4k exhibited higher binding affinity at COX-1 and COX-2 actives sites Compounds 4h and 4n exhibited higher binding affinity at PPARγ receptor protein and compound 4k exhibited higher binding affinity at COX isoenzymes' active sites in molecular docking studies. AutoDock 4.2.6 software was utilized to know the type of interactions of the designed 3D-structured thiazolidinediones with the 2PRG, 1EQG, and 1CX2 active site regions [13].

GanugapatiJayasreeand et al used AutoDock 4.0 for theDocking studies which are essential to understand the interaction between the protein and the ligands. In this case the crucial protein from Insuline receptor and ligands from Cinnamon. Docking studies are essential to understand the interaction between the protein and the ligands. In Autodock studies, the best pose was obtained with least energy value from which it can be hypothesized that these 2 compounds can be considered as potential activators of insulin receptor. After that the lab studies have to be performed to confirm the

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properties of these 2 compounds. tubulin isotypes. This indicates that select triple-modified 4 –chloro thio colchicines derivatives representhig hlypromising novel cancer chemotherapeutics. All the poses generated by both programs were rescored using the Vina scoring function. The top binding pose of colchicine and the derivatives was predicted by the Vina scoring function for the α-βII. a model which produce the best result sin terms of pIC50 predictions. Active residues involving non-hydrophobic interactions with the ligand are also specified for each compound [14].

Sarfaraz Alamand et al used AutoDockVina software (Scripps Research Chandrajit Dohutia and et al used AutoDock 4.2 to identify the receptor protein PfATP6 was the common target of artemisinin and curcumin. It was initiated to assess the antimalarial activity of six curcumin derivatives based on their binding affinities and correlating the in silico docking outcome with in vitro antimalarial screening. A ligand library of thirty two Knoevenagel condensates of curcumin were designed and docked against PfATP6 protein and six compounds with the best binding scores were synthesized and screened for their antimalarial activity against the sensitive 3D7 strain of Plasmodium falciparum [15].

Adam Huczynski and et al used AutoDock Vina and DOCK 6.5 for In silico studies to predict binding modes of the 4-chloro thiocolchicine derivatives to different β Institute, La Jolla, CA, USA) for molecular docking studies to validate the LibDock score. The designed compounds are optimized and then use for docking experiments. The docking program takes the PDBQT file format of ligands and receptor, a modified PDB file, which has added polar hydrogens and partial charges [16].

Leena K Pappachen and et al used AutoDock version 4.0 screeing program for doking the Benzothiazole derivatives with the crystallographic structures of the targets. AutoDock screening program also used to know about the hydrogen bonding interactions of all the derived compounds. The number of hydrogen bonding will considerably increase the affinity of ligand target interaction. The AutoDock shows that most of the benzothiazole derivatives show higher hydrogen bonding between the ligand-target interactions. The hydrogen bonding interactions increases the binding energy of ligand-protein interactions. The docking scores obtained for benzothiazole derivatives (BT1, BT2, BT3, BT4) and std. tamoxifen from the preliminary docking program using using AutoDock program were −6.29, −5.25, −7.19, −7.48, −3.86, r. All the four derivatives were synthesized, characterized, and subjected to in vitro anticancer screening by MTT assay in breast cancer (MCF-7) cell lines. Compounds DBT1, DBT2, and DBT3 were the most active compounds against MCF-7 cell lines with inhibitory concentration 50% of 70.0, 64.0 and 65.0, respectively. All the four derivatives show good docking scores when compared to standard drug tamoxifen and can be concluded that all the synthesized benzothiazole ligands show good anticancer property [17].

Gurudeeban Selvaraj and et al used AutoDock 4.0 for the docking analyses, There are different bonding modes of one ligand with multiple active cavities of DPP-IV. The docking analyses indicate that the bioactive constituents, β-stigmasterol, barbamide, docosahexaenoic acid, arachidonic acid, and harman shows the best binding energies on DPP-IV receptor and hydrogen bonding with ASP545, GLY741, TYR754, TYR666, ARG125, TYR547, SER630, and LYS554 residues. This result shows that docosahexaenoic acid, arachidonic acid, β-stigmasterol, barbamide, harman, ZINC58564986, ZINC56907325, ZINC69432950, ZINC69431828, ZINC73533041, ZINC84287073, ZINC69849395, and ZINC10508406 act as DPP-IV inhibitors [18].

J. Selvaraj and et al used the AutoDock softwear for validation through structure analysis verification server. For identification, the new potential drugs against GLUT4 protein the molecular docking studies of 20 natural compounds were carried out using AutoDock. The results shows that modeled structure has 87.9% residues at the core region.It was also shows that the good binding interactions of the ligand with both the targets at very low energy level. Based on the docking energy value, H-bond interaction the compounds hesperidin, fisetin, eriodictyol, wogonin, and chrysin was selected as the most potent compounds for GLUT4 protein. Hence, It was conclude that the compound shows the Anti Diabetic activity [19].

Natarajan Kiruthiga and et al used the AutoDock 4.2 for identify the binding modes of titled compounds responsible for the activity on the receptor sites. The compound HFd with 2, 4-dimethoxy group on ring C and 7-hydroxy substitution on ring A showed binding interactions with amino acid residues of alpha amylase as Arg 61, Pro 44, His 299, Gln 41 and Asp 96. Hence the scaffolds were acts as a navigator in the management of diabetic mellitus [20].

Saravanan R.R and et al used the AutoDock program for the docking simulations in the active sites of 2XNU, which shown the successfully reproduce binding modes in terms of lowest docking energy. The target protein structures of 2XNU were docked with MPIPA which shows the excellent results as were seen by the least values of the binding energy with the help of Autodock

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v4.0, In docking studies of the title derivative it understand that the possibility of these compounds to act as effective inhibitors [21].

Humaira Nadeem and et al used the AutoDock Vina softwear forThe interactions between the compounds and active site residues of H^+/K^+ ATPase. SCH28080 was used to validate the docking results. The results clearly indicate that these novel benzimidazole-pyrazole hybrids can present a new class of potential anti ulcer agents and can serve as new anti-ulcer drugs after further investigation [22].

Chandrajit Dohutia and et al used AutoDock 4.2 for receptor molecule for the docking and also study to probe the binding free energy between the ligand library and receptor. Autodock Tools (ADT) also used for optimize the receptor and ligand molecules. For preparation of the receptor molecule, polar hydrogens, Kollman charges and AD4 type of atoms were added, while Gasteiger charges were added on the ligands and maximum numbers of active torsions were given [23].

CONCLUSION:

This review totally focused on efficiency of AutoDock softwear and its different version such as AutoDock Vina, AutoDock 4.0, AutoDock 4.2.6 for molecular docking study. This information become benchmark for reseachers those uses the AutoDock softwear for study the different therapeutic activities like Antidiabetic activity, AntiMalarial activity, Antiurase activity, Anticancer activity, Antimycobacterial tuberculosis activity, Antioxidant activity, Etc. The AutoDock and its Tools are more efficient to determine the synthesis techniques, spectral analysis, docking simulation, photochemical activities, therapeutic effects, toxicological studies. These observation based on the present review have been becoming a helpful tool to guide researchers for molecular docking study. AutoDock softwear is helpful for researcher as thissoftwear is predicting docking score which is corelate with *Invivo* or *In vitro* activity. Sp this is the ultimate tool in drug discovery.

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LIPOSOME AS CARRIER FOR CANCER TREATMENT: A REVIEW

D. S. Kachare*[1], R. K. Pawar[1], P. K. Ghadge[2] and Sachin S. Mali[3]

[1]Department of Pharmaceutics, Yashoda Technical Campus, Wadhe, Satara, India.

[2,3]Department of Pharmaceutics, Y. D. Mane Institute of Pharmacy (Diploma), Kagal, Kolhapur, India.

***Corresponding Author: D. S. Kachare**

Department of Pharmaceutics, Yashoda Technical Campus, Wadhe, Satara, India.

Email id: dhanshrikachare44@gmail.com

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ABSTRACT

Liposome are risen up out of self-shaping lipid bi-layer upon hydration; liposomal medicate have job in strong medication detailing to improve therapeutics. Liposome definitions assists with diminishing poisonousness and increment aggregation at the objective site. New techniques for liposome readiness dependent on lipid medicate communication or liposome aura component. Likewise incorporate the restraint of quick freedom of liposome by diminishing molecule size, charge or surface hydration. The liposomes are portrayed by physical, substance and natural parameters. This method of medication have more security and viability to organization of a few classes of medications like antimicrobial, antiviral, antibodies, against tubercular, antifungal, medications and quality therapeutics. Utilizations of the liposomes are in the tumor treatment immunology, antibody adjuvant, eye issue, cerebrum focusing on, infective malady, dermatology. New improvements in this field is explicit restricting properties of a medication transporter of liposome to target cell as a tumor cell and explicit atoms in the body. Secrecy liposomes are particularly utilized as transporters for hydrophilic (water solvent) consumption of macrophages.

KEYWORDS: Preparations Method, Characterization, Liposome Classification, Liposomal Drugs in Cancer.

INTRODUCTION

Late improvements on the field of liposome, with malignancy treatment are principle region of intrigue. Liposomes are utilized to improve malignant growth treatment because of their ability to expand the dissolvability of inadequately hydrophilic antitumor medications. Liposomes were found around 40 years prior by A.D. Bingham. Significant enemy of tumor sedate doxorubicin figured as liposome in 1980 to improving the remedial record. Liposome definition are utilized to lessen harmfulness and increment collection at the objective site. Secrecy liposomes utilized as bearers for hydrophilic anticancer medications like doxorubicin. The liposomes are described on premise of physical, synthetic and natural parameters. These methodologies decrease tranquilize debasement and inert when its organization, they likewise increment the medication's bioavailability and the part of medication conveyed in the obsessive territory, they improving adequacy and additionally limiting medication poisonousness. Liposomes as drug carrier with great variety of molecules such as nucleotide, small drug molecules, proteins, plasmids.(Anwekar, Patel, and Singhai 2015)

DEFINATION AND STRUCTURE OF LIPOSOME

(Shashi K., 2012)

When phospholipids spread in water, they form close structure with internal aqueous environment bounded with phospholipid bilayer membranes, and create vesicular system is called as liposome. Liposome is spherical sac of phospholipid molecule enclosing water droplet as formed to carry drug or other substance into the tissue. Liposomes are spherical vesicles composed one or more lipid bilayers, involve an aqueous compartment.

Basic structure & composition of liposome

Biodegradable and biocompatible phospholipids and sphingolipids are the lipids that are most commonly used to prepare liposomes. These structural lipids can be natural or synthetic origin, natural origin consist mixture of various lipids. Cylindrical molecular-shape lipids, such as phosphatidylcholine, phosphatidylserine, phosphatidylglycerol, and sphingomyelin, are choose for liposome formulations. These lipids, are used due to their appropriate stability and their ability to act again changes in pH or salt concentrations in the product or biological environment.

Conventional liposomes possess different lipid compositions, the most commonly used lipids are cholesterol and phosphatidylcholines. Drawback of conventional liposomes is their rapid uptake by MPS after systemic administration. In 1980, development of long-circulating liposomes increases interest in the

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clinical application of liposomes as a drug delivery system for various types' cancer treatment.

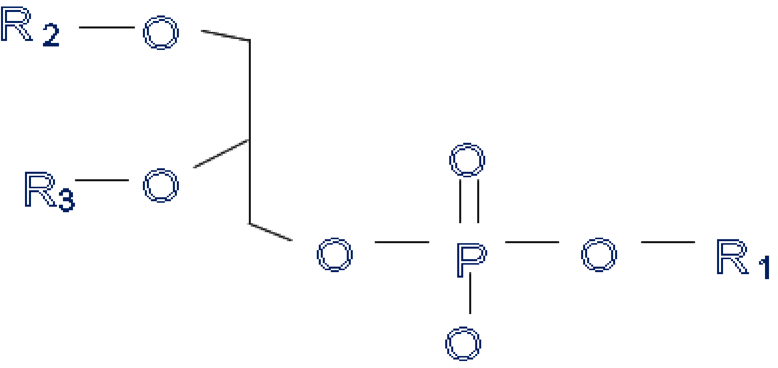

Fig. 1: Chemical Structure of Liposome.

Classification

1. Classification Based on Structure

Types of vesicle with their Dimeter Size and Number of Lipid Bilayers

Vesicle Type	Abbreviation	Diameter Size	No of Lipid Bilayer
Unilamellar vesicle	UV	All size range	One
Small Unilamellar vesicle	SUV	20-100 nm	One
Medium Unilamellar vesicle	MUV	More than 100nm	One
Large Unilamellar vesicle	LUV	More than 100nm	One

2. Based on Method of Preparation

Liposome Preparation Methods and Formed Vesicles types

Preparation Method	Vesicle Type
Single lamellar vesicle made by reverse phase evaporation	REV
Multi lamellar vesicle made by reverse phase evaporation	MLV-REV
Stable lamellar vesicle	SPLV
Frozen lamellar vesicle	FATMLV
extrusion technique	VET
Dehydration- Rehydration method	DR V

3. On basis of Composition and Application

Liposome and their Compositions

Type of Liposome	Abbreviation	Composition
Conventional liposome	CL	Negatively or neutral charge cholesterol and phospholipids
Fusogenic liposome	RSVE	Reconstituted sendai virus envelops
Cationic liposome	-	Cationic lipid with DOPE
Long circulatory liposome	LCL	Neutral high temp, cholesterol, and 5-10% PEG, DSP
Immune liposome	IL	recognition sequences or LCL with attached monoclonal antibody

4. on basis of Conventional Liposome

A. Mixtures of Stabilize natural lecithin
B. Chain phospholipids, Synthetic identical
C. Glycolipids.

5. on basis of Specialty Liposome

A. Bipolar fatty acids
B. Liposome directed by Antibody.
C. Methyl/ Methylene x- linked liposome.

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D. Lipoprotein coated liposome.
E. Carbohydrate coated liposome.
F. Multiple encapsulated liposome.(Delivery and View 2007)

Biological Properties of Liposome

1. Liposomes are biocompatible.
2. Liposomes can entrap water-soluble (hydrophilic) pharmaceutical agents in their internal water compartment and water-insoluble (hydrophobic) pharmaceuticals into the membrane.
3. Liposome-incorporated pharmaceuticals are protected from the inactivating effect of external conditions, yet do not cause undesirable side reactions.
4. Liposomes provide a unique opportunity to deliver pharmaceuticals into cells or even inside individual cellular compartments.
5. Size, charge and surface properties of liposomes can be easily changed by adding new substance to the lipid mixture before liposome preparation.

Mechanism of Liposome in Body

Liposome attaches to cell membrane and releasing their content into the cell. They are taken by the cell and their phospholipids are incorporated into the cell membrane. In the case of phagocyte cell, the liposome are taken up the phospholipid walls are act on organelles called lysosomes and the active pharmaceutical substance are released.

METHODS OF LIPOSOME PREPRATION (Lasic 1995)

At the point when phospholipids are hydrate liposome are shaped. Liposome arranged by three stages: vesicle development, vesicle size decrease, and decontamination. Arrangement techniques dependent on the creation and different contemplations, for example, tranquilize exemplification proficiency, the medication's physicochemical attributes, and the organization course. The most usually utilized strategies for liposome readiness are lipid hydration and the substitution of natural solvents by a fluid media. The lipid hydration by vortex or manual mixing, known as Bang ham's strategy.

This technique comprises of dissolving the lipids in an appropriate natural dissolvable, for example, methanol or chloroform. This procedure is trailed by expelling the dissolvable under diminished tension, revolving vanishing, until a meager film has been shaped. After, slim film is hydrated in a fluid medium, over the stage change temperature, bringing about the development of MLV liposomes. This is the least complex technique for vesicle arrangement; be that as it may, it is restricted being used on account of its low exemplification capacity. There are several groups of phospholipids that can be used for the liposome preparation which are as follows:

I. Natural source Phospholipids
II. Modified Phospholipids from natural source
III. Semi synthetic phospholipids
IV. Fully synthetic phospholipids
V. Phospholipids with natural head groups

Cholesterol can be added to the bilayers mixture for the following purposes:

1. Act as a fluidity buffer.
2. Cholesterol intercalated with phospholipids molecules they Alters the freedom of carbon molecule formation of in the acyl chain.
3. Transformation of Trans to gauche conformation is restrict.

Cholesterol decreased the permeability coefficients of positive, negative, neutral as well as negatively charged membranes to K+, Na+, Cl- and glucose. Cholesterol is necessary for lowering the membrane permeability, and imparting better stability. Cholesterol also modulates membrane-protein interactions.

Strategies for the preparation of liposomes:

1. Mechanical methods

A. Film Method

The original method is simplest procedure for the liposome formation, in this technique liposome are prepared by hydrating thin lipid film in an organic solvent then organic solvent is removed by film deposition under vacuum. When all the solvent get removed, the solid lipid mixture is hydrated by using aqueous buffer. The lipids spontaneously hydrate and swell to form liposome.

B. Ultrasonic Method

Ultrasonic method is used for the preparation of SUVs with diameter in the range of 15-25 m. Ultra-sonication of an aqueous dispersion of phospholipids is done by either probe sonicators or bath sonicators. The probe sonicators used for the small volume which requires high energy and bath sonicators are employed for the large volume.

METHODS BASED ON REPLACEMENT OF ORGANIC SOLVENTS

In that method lipids is co-solvated in organic solution, then dispersed into aqueous phase which containing material to be entrapped within the liposome. Replacement of organic solvents has two types:

A. Reverse Phase Evaporation: The lipid mixture is added in to round bottom flask and solvent is removed under reduced pressure by using rotary evaporator. This system is purged with nitrogen and lipids they re-dissolved in the organic phase. Diethyl ether and isopropyl ether is used as solvents. After re-dissolution of lipid the emulsion is obtained then the solvent is removed from emulsion by evaporation into semisolid gel under reduced pressure. Then Non encapsulated material is removed. The resulting liposomes are called reverse phase evaporation vesicles (REV). Reverse Phase Evaporation used for the preparation of large uni-lamellar and oligo-lamellar vesicles formulation and also

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it has the ability to encapsulate large macromolecules with high efficiency.

B. Ether Vaporization Method: There are two method according to the solvent used: a) Ethanol injection method. b) Ether injection method. In ethanol injection method, the lipid is inserted rapidly through a needle into an excess of saline or other aqueous medium. In ether injection method the lipid is inserted slowly through a needle into an excess of saline or other aqueous medium.

METHODS BASED ON FUSION OF PREFORMED VESICLE OR SIZE TRANSFORMATION

A. Freeze Thaw Extrusion Method: This method is an extension of the classical DRV method. Liposomes is prepared by the film method is vortexed with the solute is entrapped until the entire film is suspended and the resulted MLVs is frozen into Luke warm water and then again vortexed. After 2 cycles of freeze thaw and overtaxing the sample is thrust three times. This is followed by 6 freeze thaw cycle and addition eight thrust. This process break and defuses SUVs during which the solute equilibrates between inside or outside and liposome themselves fuse and boost in size to form large Uni lamellar vesicle by extrusion technique (LUVET). This method is widely used for the encapsulation of protein.

B. The Dehydration- Rehydration Method: In this method the empty buffer which containing SUVs and rehydrating it with the aqueous fluid containing the material is entrapped after they are dried. This leads to a dispersion of solid lipids in finely subdivided form. The vesicles are rehydrated. Oligo lamellar vesicle liposomes obtained by this method.

LIPOSOME CHARACTERIZATION (Koudelka S, Masek J, 2010)

After preparation and before use in immunoassay the liposome must be characterized. Evaluation could be classified on basis of physical, chemical and biological methods. The physical methods include parameters such as shape, surface features, size, Lamellarity, drug release profile, phase behaviors.

Methods of liposome characterization

CHARACTERISTICS	METHODOLOGY
Phospholipids quantification	Bartlett method (Lipid phosphorus content)
Lysophospholipids quantification	Bartlett method combined with Liquid chromatography
Lipid oxidation	Spectroscopy, high-performance liquid chromatography (HPLC), gas-liquid chromatography (GC), TLC,
Determination of the encapsulation percentage	Spectrophotometry, fluorescence spectroscopy, enzyme-based methods, electrochemical techniques and HPLC
Size	field-flow fractionation and analytical centrifugation, microscopy techniques (light, electronic and atomic force), Static and dynamic light scattering, size-exclusion chromatography,
Surface charge	electrophoretic mobility associated with the Photon correlation spectroscopy
Lamellarity	Nuclear magnetic resonance (^{31}P-NMR), electron microscopy, small angle X-ray scattering
Lipid phase	X-ray diffraction, differential scanning calorimetry
Phase-transition temperature	Differential scanning calorimetry and nuclear magnetic resonance (^{31}P-NMR or ^{1}H-NMR)

Biological characterization is used in establishing the suitability and safety of formulation for the in vivo application for therapeutic use. The characteristics of the carrier with appropriate choice of size, membrane components, charge determines the final behavior of liposomes in-vitro and in-vivo.

Characterization of Liposomes with their Quality Control Assays.

A. Biological Characterization

Characterization parameter	Instruments for analysis
Sterility	Aerobic/anaerobic
Pyrogenicity	Rabbit fever response
Animal toxicity	Monitoring survival rats

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B. Chemical Characterization

Characterization parameter	Instruments for analysis
Phospholipid Concentration	HPLC / Barrelet assay
Cholesterol Concentration	HPLC / Cholesterol oxide assay
Drug Concentration	Assay method
Phospholipid peroxidation	UV observance
Phospholipid hydrolysis	HPLC / TLC
Cholesterol auto oxidation	HPLC / TLC
Antioxidant degradation	HPLC / TLC
PH	PH meter
Osmolarity	Osmometer

C. Physical Characterization

Characterization parameter	Instruments for analysis
Vesicle shape and surface morphology	TEM and SEM
Vesicle Size and size distribution	Dynamic light scattering, TEM
Surface Charge	Free flow Electrophoresis
Electrical surface potential and surface PH	Zeta potential measurement
Lamellarity	P^{31} NMR
Phase behavior	DSC, Freeze facture electron microscopy
Percent capture	Gel exclusion
Drug release	Diffuse

Stratergies to Optimize Liposome Stability (Abdelwahed W, 2006)

Business item including in liposome plan contain security attributes and timeframe of realistic usability over one year. It is presently conceivable to acquire a reproducible readiness of enormous volumes of stable liposomes and long haul security issues have additionally been effectively explained.

Security of liposomes is significant part in their improvement for pharmaceutical applications. Potential utilization of liposomes as helpful instruments is tested by their inborn synthetic and physical shakiness in water mediums, they can bring about expanded bilayer porousness and vesicle combination, resulting drug spillage and precipitation. Dangers of liposome is invigorated by bilayer surrenders instigated by synthetic debasement and by physical factors(heating or freezing) because of stage advances happen when these fluid scatterings is put away for expanded periods. The significant method to support liposome dependability is to get ready suitable plan, which help for the choice of the proper lipid fixation and organization, also the expansion of different substances for improve its timeframe of realistic usability. Item that can assists with expanding liposomal soundness include lyophillization and proficient detailing. Definition includes the choice of the appropriate lipid sythesis, centralization of bilayers, cancer prevention agent, and watery stage fixings as supports, cryo protectants and metal chelators. Charge including lipid as phosphotidyl glycerol consolidate into liposome bilayers to lessen combination while cholesterol and sphingomyelin can be remembered for the detailing to diminish penetrability and spillage of epitomized substance. Supports at impartial pH help to diminish hydrolysis; expansion of cancer prevention agent, for example, sodium ascorbate can decrease oxidation. In general, formulation of stable liposomal drug product requires the following precautions:

1. Processing with purified, fresh lipids and solvents.
2. Avoidance of high temperature and excessive shear forces.
3. Maintenance of low oxygen potential.
4. Use of metal chelators and antioxidant.
5. Formulating at neutral pH.
6. Lyo protectant used for freeze drying.

LIPOSOME IN CANCER THERAPY (Hofheinz RD, 2005)

The drawn out treatment of anticancer medication prompts a few harmful reaction. The liposomal treatment for the focusing to the tumor cell have been altered the universe of malignancy treatment with least reaction. It has been said that the little and stable liposome are inactively focused to various tumor since they can circle for longer time and they can extra vasate in tissue with improved vascular penetrability. Liposome macrophage take-up by liver and spleen hampered the improvement of liposome as medication conveyance for more than 20 years.

Liposomes have been utilized as bearers of platinum mixes (cisplatin and oxaplatin), anthracyclines (doxorubicin and daunorubicin), paclitaxel, camptothecin subordinates, antimetabolites (methotrexate, cytarabine), and Vinca alkaloids (vincristine, vinblastine and vinorelbine), planned for decreasing the poisonous reactions of cytostatic drugs without hampering their viability. Their applications depend on the capacity of liposomes to change the tissue dispersion of the ensnared tranquilize, which gets reliant on the physicochemical highlights of the liposomes and not the embodied

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substance. In malignant growth chemotherapy, the uninvolved focusing of liposomes exploits the inborn size of nanoparticles and the exceptional properties of tumor vasculature. As tumors develop and exceed the accessible flexibly of oxygen and supplements, they discharge particles that enroll fresh blood vessels to the tumor in a procedure called angiogenesis. Not at all like the tight veins in typical tissues, angiogenic veins in tumor tissues contain holes as extensive as 600 to 800 nm between contiguous endothelial cells.

This nature of tumor angiogenesis, combined with poor lymphatic waste, prompts an upgraded penetrability and a maintenance impact (EPR). Thusly, long-circling liposomes will specially extravasate from these anomalous vessels and can specifically gather inside the tumor interstitiumty or common capacity to target malignant growth. The endothelial dividers of all sound human vein are embodied by endothelial cells limited together by close intersections. These tight intersections help to stop the enormous molecule in blood from spilling out of the vessel. Such sort of plan isn't there in the event of tumor vessel and subsequently is demonstratively "cracked". This capacity is knownas upgraded penetrability and maintenance impact. Liposomes of size under 400 nm, can quickly enter tumor locales from blood, however these are then kept in circulatory system by endothelial divider in solid tissue.

Platinum compound: (Mishra 2004)

Cisplatin (CDDP) is one of the most effective chemotherapeutic agents used by intravenous route in the treatment of ovary, lung, testicle, head, and neck carcinomas. Furthermore, CDDP has been widely used in the treatment of peritoneal carcinomatosis by intraperitoneal route. However, the administration of CDDP by both routes is still hindered by toxicity, mainly nephrotoxicity. Conventional liposomes composed of phosphatidylcholine/ phosphatidylserine/ CHOL containing CDDP were evaluated in IgM immunocyto bearing LOU/M rats.

An increased tumor platinum uptake and a significantly improved antitumor effect could be observed with the use of SPI-077, as compared to free CDDP. Another long-circulating liposomal formulation containing CDDP made up of soy phosphatidylcholine (SPC)/ DPPG /CHOL/DSPE-PEG2000 is called Lipoplatin®. This formulation was developed to reduce the systemic toxicity of CDDP while simultaneously improving the targeting of the drug to the primary tumor and metastasis by enhancing the circulation time in body fluids and tissues. Cytotoxicity studies of this formulation were performed in cell lines derived from non-small cell lung cancer, renal cell carcinoma, and in normal hematopoietic cell precursors.

Lipoplatin®, when compared to CDDP, produced a stronger cytotoxic effect in both evaluated tumor cells lines and a lower toxicity in normal bone marrow stem cells. Antitumor efficacy of Lipoplatin® was assessed in xenografts of human breast, prostate, and pancreatic cancer, where a reduction in tumor size could be observed. Measurement of platinum levels in the plasma of patients as a function of time showed that a maximum platinum level is attained at 6-8 hrs. The half-life of Lipoplatin® was 60-117 hrs, depending on the dose. The determining of platinum levels in excised tumors and normal tissues showed that Lipoplatin® has the ability to preferentially concentrate on the malignant tissue (10-50 fold) of both primary and metastatic origin.

The pharmacokinetic profile of Lipoplatin® in combination with 5-fluorouracil showed that the liposomal formulation has a greater body clearance and a shorter half-life than does free CDDP, which confirms the clinical observation of decreased toxicity, especially nephrotoxicity. Phase I, II, and III trials have shown that Lipoplatin® presents similar antitumor efficacy to CDDP in pancreatic, head and neck, breast cancers, and non-small cell lung carcinoma, as well as reduced toxicity, nephrotoxicity. Although CDDP is one of the most widely used chemotherapeutic agents, the development of tumor cell resistance against CDDP is a limitation in the clinical application of this drug. The lipid composition of liposomes contained SPC/CHOL/distearoyl phosphatidyl ethanol aminepolyethyl eneglycol (DSPE-PEG). CDDP-containing liposomes were prepared, and the target ability of transferrin receptors (TfR) to correlate CDDP cell uptake with cytotoxicity in sensitive and CDDP resistant ovarian cancer cells.

These liposomes were stable in plasma, circumvented the preclinical resistance to treatment with CDDP. CDDP has also been widely used in the treatment of peritoneal carcinomatosis by the intraperitoneal route. However, CDDP, a low-molecular-weight compound, is rapidly absorbed by the capillaries in the i.p. serosa and transferred to the bloodstream, inducing the appearance of systemic side-effects, such as nephrotoxicity.

Oxaliplatin an analoge of CDDP, has shown a good in vitro and in vivo antitumor effect and a better safety profile than cisplatin. However, the use of oxaliplatin is associated with side-effects which include neurotoxicity, hematologic toxicity and gastrointestinal tract toxicity. Lipoxal® is a liposomal formulation of oxaliplatin made up of hydrogenated soy phospahatidylcholine (HSPC)/DPPG/ CHOL/DSPE-PEG. This liposomal formulation containing oxaliplatin has also proven to induce the complete disappearance of human breast cancers in mice after 6 intravenous injections with 4 days intervals at a dose of 16 mg/Kg. On the other hand, the free oxaliplatin at its MTD could only cause shrinkage, not the disappearance of tumors.

Anthracyclines compound: (Leonard RCF, 2009)

The anthracyclines, represented by doxorubicin, daunorubicin, and their derivatives (Figure 9), are widely

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used in the treatment of several hematological and solid tumors and are considered to be a first-line therapy for advanced breast cancer [100]. Although conventional anthracyclines have been extensively used for the treatment of a variety of cancers.

Cardiotoxicity can be increased nearly four-fold when these drugs are administered in association with other chemotherapeutic drugs. Ability of liposomes containing daunorubicin (DNR), made up of DSPC/CHOL.

Another commercial product of conventional liposome (Myocet®), in combination with cyclophosphamide, has been approved in Europe as a first-line treatment of breast cancer. This liposome consists of egg phosphatidilcholine (EPC)/ CHOL and encapsulated doxorubicin (DXR). The ability of Myocet® to locate tumors could be observed in ascitic (L1210 ascitic lymphoma). Doxil®/Caelix® was the first and is still the only long-circulating liposome formulation to be approved in both the USA and Europe to treat Kaposi's sarcoma and recurrent ovarian cancer. Indicating that the pharmacokinetics of liposomal DXR is governed by the liposome carrier and that most of the drug is delivered to the tissues in liposome-associated form [115]. Several studies are currently in progress using Doxil®/Caelix® to treat other malignancies, such as breast cancer and recurrent high-grade glioma.

Other chemotherapeutic agents

Another important drug in cancer therapy is paclitaxel. This is an alkaloid which stabilizes microtubules and inhibits endothelial cell proliferation, motility, and tube formation. Some studies have presented difficulties in the development of liposomes containing paclitaxel due to its hydrophobic nature. Therapeutic efficacy studies performed in a mouse xenograft model of human ovarian (OVCAR-3), human lung (A-549), breast (MX-1), and prostate (PC-3) cancer, as compared to the administration of free drugs, demonstrated greater tumor growth inhibition after the administration of liposomal paclitaxel. In addition, toxicology studies have shown that liposomal paclitaxel is less toxic than free paclitaxel. An improved pegylated liposomal formulation of paclitaxel was developed, demonstrating that cytotoxicity in human breast cancer cell lines (MDA-MB-231 and SK-BR-3) of the tested paclitaxel formulation was equipotent after 72 hrs of incubation, when compared to Taxol®.

RECENT ADVANCES IN TARGETED LIPOSOME

(Sawant RR, 2012)

In an attempt to improve the binding and cellular internalization of liposomes in the tumor area, several ligands were attached to the liposome surface, including monoclonal antibodies, folate, transferrin, vasoactive intestinal peptide (VIP), epidermal growth factor (EGF), hyaluronan, galactosides, and condroitin sulphate.

The majority of research in this area is related to cancer targeting, which uses a variety of monoclonal antibodies. To target HER2-overexpressing tumors, it was suggested that antiHER2 long-circulating liposomes be used. Nucleosome-specific monoclonal antibody (maybe 2C5) capable of recognizing various tumor cells through the tumor cell surface-bound nucleosomes significantly improved Doxil®, by targeting to tumor cells, and increased its cytotoxicity both in vitro and in vivo in different testing systems, including the intracranial human brain U-87 tumor xenograft in nude mice. The same antibody was also used to effectively target long circulating liposomes loaded with an agent for tumor photodynamic therapy (PDT) for both multiple cancer cells in vitro and experimental tumors in vivo, and provided a significantly enhanced elimination of tumor cells under PDT conditions.

Saccharide molecules represent good models for tumor targeting molecules, as many malignant cells express the lectin, sugar-binding protein. They concluded that disaccharide-modified liposomes may be promising cancer targeting carriers which can enhance intracellular uptake and cytotoxicity of the drug-loaded liposomes by means of lectin-mediated endocytosis.

One approach that has received considerable attention has been the use of folic acid to deliver drugs selectively to folate receptor-expressing cancer cells. Studies of folate-conjugated liposomes containing DNR or DXR showed an increased cytotoxicity of the encapsulated anticancer drugs in various tumor cells. The i.v. administration of anti-tumorassociated glycoprotein (TAG)-72 Polyethyleneglycol (PEG)-immunoliposomes showed that they were more effectively located in LS174 T human colon cancer cells than conventional liposomes.

FUTURE PERSPECTIVE AND CHALLENGE

(Cukierman and Khan 2010)

The more prominent enthusiasm for the advancement of these complex medication conveyance frameworks is to improve the adequacy and decline the symptoms of new and old enemy of malignant growth drugs. In this specific circumstance, the advanced pharmacokinetic properties of liposomes, bringing about an improved harmfulness profile, is as yet the principle contention for the utilization of liposomal transporters. Other new methodologies in the science and pharmacokinetic conduct of liposomes, for example, the counter angiogenic properties of cationic liposomes, just as the advancement of immunoliposomes or antisense oligonucleotides, likewise offer an incredible restorative collection for these medication conveyance frameworks. There are numerous issues with respect to the shakiness of particles through flocculation and total, their mind boggling stream, and bond designs in the slender system, the heterogeneity of the entrance of medications to explicit tumor locales, the dispersion of free medications, and nanoparticles in tumor tissues just as in single cells.

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Many research bunches are taking a shot at progressively "dynamic" treatments that adventure focusing on ligands to convey joined cytotoxic medications specifically to threatening cells. These ligands explicitly perceive and specially tie receptors found on the cells of intrigue, subsequently taking into consideration an increasingly exact conveyance technique. Albeit current investigations have indicated that the utilization of these focused on nanoparticles as a medication conveyance framework is a promising methodology to treat human malignant growths, it is still in its beginning time of advancement. Clinical information utilizing focused on nanoparticles are constrained, since most focused on nanoparticles have not yet arrived at the clinical level.

APPLICATION OF LIPOSOME (Dua J.S, 2012)

1. Enhance drug solubilisation (Amphotericin-B, Minoxidil, Paclitaxels, and Cyclosporins)
2. Protection of sensitive drug molecules (Cytosine arabinosa, DNA, RNA, Anti-sensoligo-nucleotides, Ribozymes)
3. Enhance intracellular uptake (Anticancer, anti-viral and antimicrobial drugs)
4. Altered pharmacokinetics and bio-distribution (prolonged or sustained released drugs with short circulatory half-life).

Liposomes can be utilized additionally to convey drugs into the lung. This is frequently done by inward breath of liposome vaporized. This can be utilized either for the treatment of different lung issue, diseases, asthma, or utilizing lungs as a medication stop for the fundamental conveyance. By fitting lipid sythesis an assortment of discharge energy can be acquired. One of the potential uses of these pressurized canned products is in the asthma alleviation wherein the dosing recurrence can be significantly diminished and single inward breath can last expedite. Oral uses of liposomes are at present fairly restricted due to the very liposomicidal condition in stomach and duodenum and regularly the organization of free or liposome epitomized sedate displays ordinarily no distinctions. Intragastrical organization, in any case, shows that liposomes upgrade the foundational bioavailability of certain water insoluble medications and nutrients.

A few structures to balance out liposomes in low pH, degradative catalyst, and bile salts containing situations are being contemplated. Improved solvency of lipophilic and amphiphilic drugs. Models incorporate Porphyrins, Amphotericin B, Minoxidil, a few peptides, and anthracyclines, separately; besides, sometimes hydrophilic medications, for example, anticancer operator Doxorubicin or Acyclovir can be epitomized in the liposome inside at focuses a few crease over their fluid solvency.

This is conceivable because of Precipitation of the medication or gel development inside the liposome with suitable substances typified Passive focusing to the cells of the safe framework, particularly cells of the mononuclear phagocytic framework (in more established writing reticuloendothelial framework). Models are antimonies, Amphotericin B, porphyrins and furthermore immunizations, Immunomodulators or immunosupressors. Supported discharge arrangement of fundamentally or privately controlled liposomes. Models are doxorubicin, cytosine arabinose, cortisones, natural proteins or peptides, for example, vasopressin. Site-evasion instrument: liposomes don't arrange in specific organs, for example, heart, kidneys, mind, and sensory system and this lessens cardio-, nephro-, and neuro-harmfulness. Run of the mill models are decreased nephrotoxicity of Amphotericin B, and diminished cardiotoxicity of Doxorubicin liposomes.

Site specific focusing: in specific cases liposomes with surface connected ligands can tie to target cells ('key and lock' instrument), or can be conveyed into the objective tissue by neighborhood anatomical conditions, for example, flawed and gravely shaped veins, their basal lamina, and vessels. Models incorporate anticancer, hostile to contamination and against inflammatory drugs.

CONCLUSION

This undertaking presumed that liposome as transporter which are the most generally utilized medication nanoparticles in malignancy medicines. Essential ideas were introduced concerning liposomes and a review of the clinically utilized and tried liposomes for the treatment of malignancy. It has been illustrated, in view of earlier examinations, that liposomes offer wellbeing and adequacy when contrasted with other customary medications. Liposome framework is to improve viability and abatement symptom of new and old enemy of malignant growth drugs.

The new advancements in the liposome are the particular restricting properties of a medication conveying liposome to an objective cell (tumor cell and explicit particles), secrecy liposomes for focusing on hydrophilic (water dissolvable) anticancer medications like doxorubicin, mitoxantrone which prompts decline in symptoms on the grounds that the medication is for the most part amassed at the site of activity. Other advancement is bisphosphonate-liposome intervened consumption of macrophages. A few business liposomes have just been found, enrolled and presented with extraordinary achievement in pharmaceutical market. There is much more noteworthy guarantee in future for promoting of progressively complex and profoundly balanced out liposomal details. In future the liposomal tranquilize conveyance framework will reform the vesicular frameworks with wide application particularly in the treatment of malignant growth.

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Role of Citrus Pectin in Biological Activity: A Review

D.S. Kachare*[1], P. K. Ghadge[2], Sachin S. Mali[3]
Researcher Student[1], Assistant Professor[2], Principal[3]
Department of Pharmacology[1, 2], Department of Pharmaceutics[3]
Yashoda Technical Campus, Wadhe, Satara, India[1, 2],
Y. D. Mane Institute of Pharmacy (Diploma), Kagal, Kolhapur, India[3]
Corresponding Author's email id: *dhanshrikachare44@gmail.com*[*1]

Abstract

Pectin is a naturally occurring biopolymer. It has been used successfully for many years in the food and beverage industry as a thickening agent, a gelling agent and a colloidal stabilizer. In this review, study of the role of pectin polysaccharides, including its various pharmacological activity, such as its immuno-regulatory, anti-inflammatory, hypoglycemic, antibacterial, antioxidant and antitumor activities, have been summarized. The review provides natural sources, chemical structures, biological activities, and practical applications in the food industry as well as pharmacology and different branches of medicine. Pectin has become an essential part of the research and development of natural herbs and health products due to their wide availability.

Keywords: *- Citrus Pectin, Source, extraction, Biological activity, glycogen regulation.*

INTRODUCTION

French chemist Louis Nicolas Vauquelin in 1790 in tamarind fruit, discovered pectin in citrus fruits (Vauquelin, 1790). Henri Braconnot introduced the term "pectin" because of the gelling properties of these substances (Braconnot, 1825). Pectin is usually obtained from the residues of plant material after extracting the juice (apple or citrus peel) or sugar (sugar beet). This review will first describe the source and production, chemical structure and general properties of pectin. Pectin is the methylated ester of polygalacturonic acid, contains 1, 4-linked D-galacturonic acid residues.

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STRUCTURE OF PECTIN

Pectin is a polysaccharide with a core consisting of α-1,4-linked D-galacturonic acid and α-1, 2-L-rhamnose, large number of neutral sugars, including arabinose, galactose, and lesser amounts of other sugars. The structural classification of pectin includes: homogalacturonan (HG), rhamnogalacturonan I (RG-I), and substituted galacturonans such as rhamnogalacturonan II (RG-II) (Figure 1). It composed of as many as 17 different monosaccharides and more than 20 different linkages. (Minzanova, Salima T et al. 2018)

GENERAL PROPERTIES OF PECTIN

Pectins are soluble in warm water. Monovalent cation salts of pectinic and pectic acids are usually soluble in water; di- and trivalent cations salts are weakly soluble or insoluble. Dry powdered pectin, when added to water, has a tendency to hydrate very rapidly, forming clumps.

Most important use of pectin is based on its ability to form gels. HM-pectin forms gels with sugar and acid. HM-pectin, unlike LM-pectin, does not contain sufficient acid groups to gel or precipitate with calcium ions, although other ions such as aluminium or copper cause precipitation under certain conditions. (Rolin C 1993), (Hercules Incorporated 1999). (Raj et al. 2012)

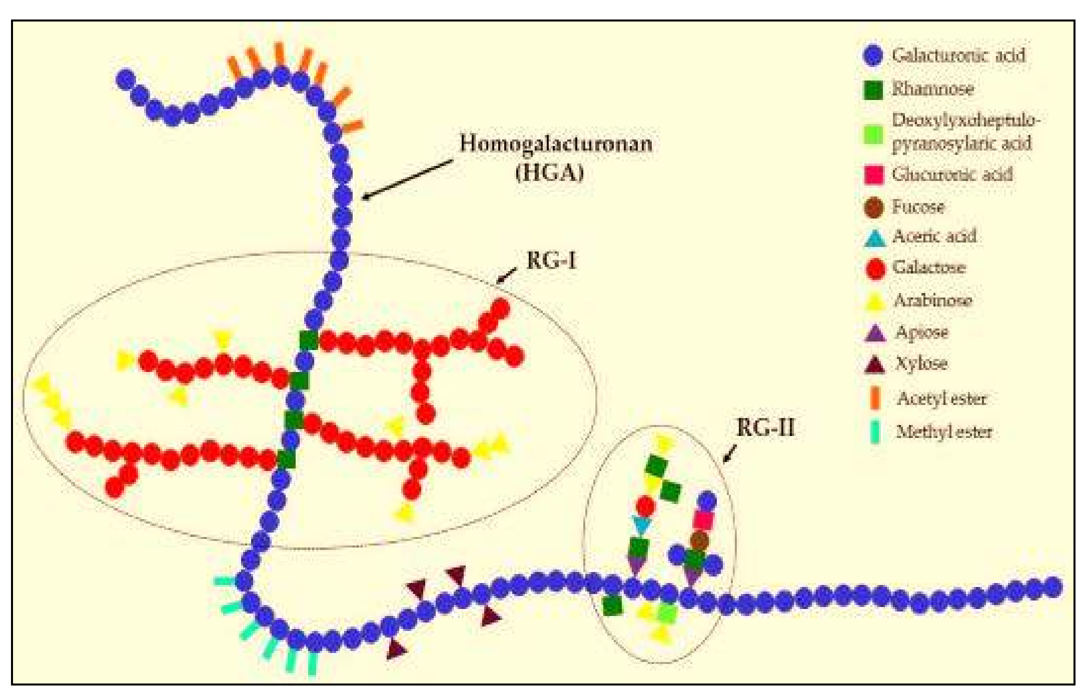

***Fig.1**. A scheme of primary structure of pectin*

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METHODSOF PECTIN PREPRATION

Sample Preparation

The samples (Cola milleni, Irvingia gabonensis and Theobroma cacao) peel and husk were sun dried for one week and making powder using blender. The powdered samples were sieved with a fine mesh of size 14mm. The sieved samples were kept in an air tight container prior to extraction process.

Extraction of Pectin (Oloye, 2013)

Extraction of pectin from the samples was performed under acid condition. The dried powder pectin was extracted by mixing with acidified distilled water inside a water bath at different temperatures ranges from 50 - 100°C and different time from 30 – 150 mins. After contact time reflux, the samples were filtered through cheesecloth and cooled; it was then centrifuged for 20 mins at 3,500 rpm. Ethanol 96% was added to the supernatant and allowed to stand for one hour for pectin precipitation. The precipitated pectin was separated by filtration, washed thrice its volumes with absolute ethanol and washed twice with water to remove impurity. The extracts were separately dried in an oven at 50°C and the pectin yield was determined. The dried pectin samples were stored in aluminium foils at 4°C until used.

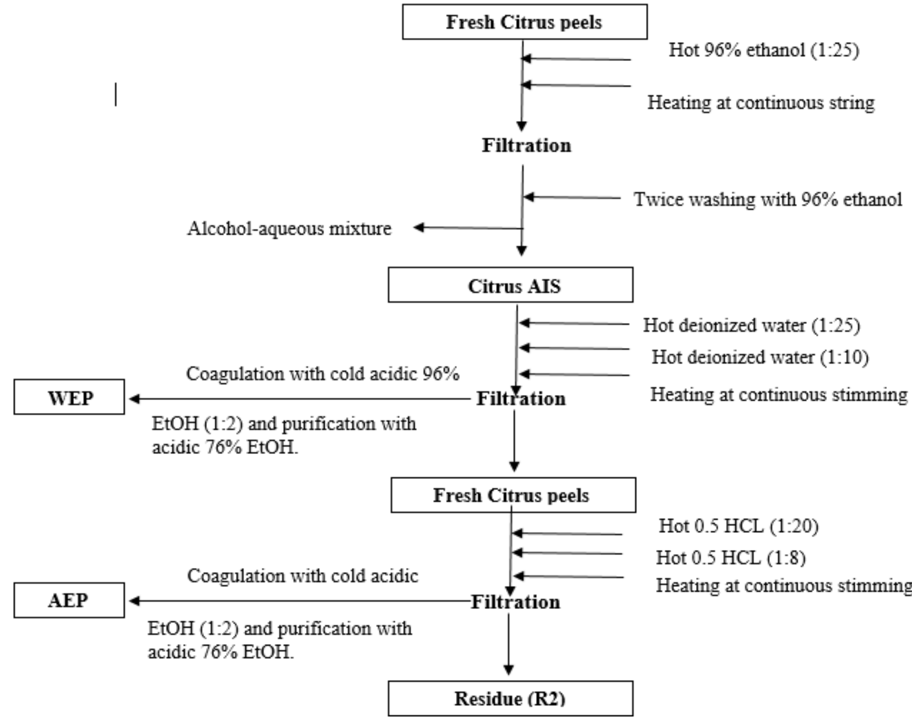

Fig.2. Preparation of pectin extraction from orange and lemon peels

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Chemical Constituents: The neutral sugars that essentially form the side chains on the pectin molecules are namely: galactose, arabinose, xylose, and fructose. They also contain Saponin, Tannin, Alkaloid, Flavonoid, Steroid, Terpenoid, Glycoside and Phenol. (Oloye, 2013)

ROLE OF CITRUS PECTIN IN BIOLOGICAL ACTIVITY

Immunomodulatory Activity:

Immunomodulators are natural or synthetic substances that have a regulating effect on the immune system. Plant-derived polysaccharides, including pectin can directly activate the immune function of macrophages, promote the production of cytokines, and therefore regulate the immune system on multiple levels. The immunomodulatory activities of the oligomer fractions are still observed in the studies. Enzymatic digestion of pectin, which also show the value of the back bone of pectin.

The carbohydrate chain of pectin determines immunosuppressive activity. It was found that pectin containing more than 80% of galacturonic acid residues suppress the activity of macrophages and inhibit the delayed-type hypersensitivity reaction. In addition, the branched region of the pectin macromolecule mediates the stimulation of phagocytosis and increased production of antibodies. (Popov & Ovodov, 2013)

Anti-inflammatory Effect:

Inflammation has been considered as a main risk factor for different progressive illnesses in human beings, such as neurological disorders, cancer, metabolic diseases, and cardiovascular disease, and a primary strategy to prevent these diseases is to target the reduction of chronic inflammation. (Leivas C.L et al., 2016)The intake of dietary fibers such as plant cell wall polysaccharides enhances the efficiency of treatment of inflammatory processes. Much attention has been focused in recent years on pectin.

Popov et al. has studied the anti-inflammatory activity of citrus pectin in vivo after oral administration in mice. (Popov S.V et al., 2013)Three models of inflammation were used: cytokine production by blood leukocytes in response to lipopolysaccharide, acetic acid-induced colitis, and endotoxin shock.

The results of the study demonstrate that low methyl-esterified citrus pectin inhibits local and systemic inflammation, while

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pectin with a higher degree of esterification can inhibit intestinal inflammation.

Antioxidant Activity:

Oxidation is vital to plenty of organisms that can generate energy to supply biological processes. In normal circumstances, free radicals govern cell growth, and suppress viruses and bacteria. Nevertheless, in large quantities and without regulation, the production of free radicals induced by oxygen cause cell damage, which renders the pathological progressions. The oxidative stress is associated with chronic obstructive pulmonary disease, asthma, diabetes, inflammation, cardiovascular diseases, and myocardial infarction. (Wang, J et al., 2016)

Antitumor Activity:

Many in vitro and in vivo studies concerning the antitumor activity of native and modified pectin revealed a decrease of adhesion and cell proliferation, as well as the induction of apoptosis and migration. (Bush, P et al., 2014) Maxwell et al. have assessed pectin from different sources (potato, sugar beet, larch, and citrus) for effects against colon cancer cells. (Maxwell,E.G et al.,2015) Extracts of potato pectin lowered the proliferation of colon cancer cells by the alteration of dose.

Sugar beet pectin extracts presenting various structures of pectin showed high anti-proliferative action against colon cancer cells. (Maxwell, E.G et al., 2016) The alkali treatment of pectin surged the antitumor activity of sugar beet pectin due to an apoptosis promotion. The pectic polysaccharide from apple can induce the death of cancer cells death and suppress the growth of tumors in vivo, as Delphi and Sepehri described. (Delphi, L et al., 2016)

Hypoglycemic / Anti-diabetic Effect:

Research was conducted to study the anti-diabetic effect of citrus pectin in diabetic rats and the potential benefits of citrus pectin to produce anti-diabetic effects in cases of T2DM caused by a low-dose streptozotocin and a fat-laden diet. Many tests have demonstrated that anti-diabetic polysaccharides effectively improve glucose tolerance.

Low-dose citrus pectin (500 mg/kg bw per day), the medium-dose citrus pectin (1000 mg/kg bw per day) and the high-dose

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citrus pectin (2000 mg/kg bw per day) intragastrically administered.

Citrus pectin reduced fasting blood glucose levels, benefited hyperlipidemia, and refined hepatic glycogen content glucose tolerance in the diabetic rats. Citrus pectin modulated the expression of the basic proteins in the PI3K/Akt signaling pathway, which could have influenced the enhancement of insulin sensitivity in the diabetic rats, possibly by signifying the anti-diabetic effect of citrus pectin. (Liu, Dong, Yang, & Pan, 2016)

Neuroprotective Effect:

We investigated the neuroprotective activities of ginseng pectin (GP) against hydrogen peroxide (H2O2)-induced neuronal toxicity in different neuronal cells. GP protects neuronal cells from hydrogen peroxide-induced cell death. GP protects cortical neuron cell neurites from degeneration. GP neuroprotective effect occurs through the activation of ERK/MAPK and Akt survival signaling pathways. Ginsenoside Rb1 has been shown to protect neuronal cells against hydrogen peroxide-induced cell damage possibly by scavenging free radicals, inhibiting the production of nitric oxide, preventing lipid peroxidation and avoiding decrease in SOD activity.

GP showed similar protective effects on neuronal cells against H2O2-induced oxidative stress via regulating the pro-survival ERK/MAPK and Akt pathways. Moreover, GP preserved the structural integrity of neurons, suggesting that it may be a new neurotrophin. In conclusion, GP appears to be anti-oxidant without side-effects, which may eventually lead to further development of therapeutics for neurodegenerative diseases. (Fan et al., 2012)

Glycogen regulation

Pectin decreased PKC (protein kinase-c) activity in liver and increased PKC activity in brain. (Kramer HK, 1997) Pectin also enhanced glycogenesis and reduced glycogenolysis. Activation of PKC stimulate the serotonin receptor or transporter in brain. (Vijayalakshmi, 2014).MCP prevents blood-brain barrier disruption possibly by inhibiting galectin-3.

DISCUSSION

Pectin structure provide significant immunomodulatory properties. However, future research studies necessary to verify

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the immune activity in vivo and determine howthemechanismsofpectinaffectmacrophagesandotherimmunocytesforsafeclinical applications.

Studies of the antimicrobial properties of pectins show a general tendency for the development of nanocomposites and nanoemulsions on their base. Both exhibit noticeable inhibitory effects, mainly against Gram-negative Escherichia coli and Gram-positive Staphylococcus aureus. The analysis of the reviewed studies shows that pectin polysaccharides from various source demonstrate antioxidant activity. It was also shown that pectins of diverse chemical structure (HG, RG-I, RG-II) exhibit antioxidant properties. Hypoglycemic activity of pectins is useful for the development of low-toxicity antidiabeticagents.

Drugs based on dietary and medicinal plants don't cause side effects, which helps pectin promising for further research. Anti-inflammatory properties of pectin is given great attention in the literature, because pectin have a great potential for anti-inflammatory multi-purpose therapy. Another fast-growing field of pectin useful application is anti-cancer therapy, which is due to safety of pectin and its derivatives. However, the lack of research on pectin polysaccharide protection mechanisms and clinical trials has limited the application of pectin in the field of medicine thus far.

CONCLUSION

Recently updated knowledge about Citrus pectinplay beneficial role in various biological activity. Pectin extracted from Citrus fruits, Cola milleni, Irvingia gabonensis and Theobroma cacao. Pectin also enhanced glycogenesis and reduced glycogenolysis .Results of a study of the bioactivity of pectin polysaccharides, including its various pharmacological action, such as its immunoregulatory, anti-inflammatory, hypoglycemic, antibacterial, antioxidant and antitumor activities, Neuroprotective have been summarized.

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Open Access **Review Article**

A Review on Herbal Medicinal Plant for Treatment of Polycystic Ovarian Syndrome (PCOS)

Karishma Yadav*[1], Priyanka Ghadge[2], Aryan Langeh[1], Shankar Kalbhare[3], Prajkta Phadtare[1], Rupali Bhoite[1]

[1]Department of Pharmacology, YSPM's Yashoda Technical Campus, Wadhe, Satara, India.
[2]Head of Department (Pharmacology), YSPM's Yashoda Technical Campus, Wadhe, Satara, India.
[3]Department of Pharmaceutics, YSPM's Yashoda Technical Campus, Wadhe, Satara, India.

ABSTRACT

Infertility due to polycystic ovarian syndrome (PCOS) is a worldwide problem that is increasing at alarming rates. It is characterized by chronic anovulation, polycystic ovaries, and hyperandrogenism leading to symptoms of irregular menstrual cycles, hirsutism, acne and infertility. Insulin resistance and elevated levels of male hormones (androgens) are associated with PCOS. The sedentary lifestyle, lack of exercise and dietary variations and stress etc., are also the contributory factors. Various plants like Panax ginseng, Punica granatum, Curcuma longa, Cinnamomum zeylanicum Tribulus terrestris, Symplocos racemosa, Trigonella foenum-graecum, Cocus nucifera etc., proved active in the treatment of PCOS. In this review, attempts have been made to summarize the important medicinal plants which are used in treatment or prevention of PCOS. Special attention is given to the role of insulin resistance and the potential utility of insulin sensitizers in management of PCOS.

Keywords: Polycystic ovarian syndrome (PCOS), Pathophysiology of pcos, Screening methods of pcos,

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***Address for Correspondence:**

Karishma Yadav, Department of Pharmacology, YSPM's Yashoda Technical Campus, Wadhe, Satara, India.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common female endocrine disorders. It is recognized by the presence of enlarged ovaries with multiple small cysts and a hypervascularized androgen secreting stroma. The clinical symptoms include menstrual abnormalities, polycystic ovary, obesity, infertility, hairy, acne, and hyperandrogenism[1]. The polycystic ovary syndrome is a clinical diagnosis characterized by the presence of two or more of the following features: chronic oligo-ovulation or an-ovulation, androgen excess and polycystic ovaries[2].

World Health Organization (WHO) estimates that PCOS has affected 116 million women (3.4%) worldwide in 2012[3]. Polycystic ovary syndrome (PCOS) is a heterogeneous disorder of unknown etiology affecting 5%-10% of women of reproductive age[4]. Globally, prevalence estimates of PCOS are highly variable, ranging from 2.2% to as high as 26%. In India, experts claim 10% of the women to be affected by PCOS[5]. PCOS are strongly interlinked by the pathogenesis of various individual disorders; they may be broadly classified into endocrine dysfunction, reproductive dysfunction, metabolic dysfunction and biochemical dysfunction[6]. It is also associated with psychological impairments including depression and other mood disorders. Most women with PCOS are also overweight or obese, further enhancing androgen secretion while impairing metabolism and reproductive functions and possibly favoring the development of the PCOS[7]. Metabolic abnormalities such as dyslipidemia, insulin resistance, therefore, diseases including diabetes, obesity, cancer and infertility as well as coronary heart diseases could be seen along with PCOS[8]. Reactive oxygen species and antioxidants remain in balance in normal individual but when this balance is disturbed, oxidative stress develops[9]. Which may lead to different disorders. Increased oxidative stress contributes to the risk of cardiovascular disease in women with PCOS[10].

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Risk factor[11]

- Family history of PCOS
- Family history of diabetes
- Family history of infertility
- Obesity
- Fast food diet habits
- Lack of physical exercise
- Stress

PATHOPHYSIOLOGY OF PCOS

The Gonadotropic releasing hormone is secreted or synthesized Luiteinsing hormone (LH) and Follicle stimulating hormone (FSH). A less amount of intraovarian androgen is use for normal follicular growth. The follicular development provides by FSH and stimulating aromatase enzyme it promotes granulose cell conversion of androgen to oestrogen. LH is initiate of oocyte maturation by inducing theca cell production.

PCOS condition increases LH level and decreasing level of FSH causes to produce more androgen and reduced level of aromatase enzyme with immature follicle development. Excessive androgen in PCOS is related of abdominal fat that leading to Hyperinsulinemia and Dyslipidemia. Increase theca cell androgen production, Hyperinsulinemia reduces sex hormone binding globulin (SHBG) to increases circulating testosterone levels[12].

Screening Methods of Pcos

Androgen-Induced Rodent Models of PCOS[13]:

Hyperandrogenism is the primary manifestation of PCOS. One etiologic hypothesis of PCOS is that exposure to excessive androgens early in life leads to PCOS in adult hood. It was reported more than 3 decades ago that elevated concentrations of circulating androgens in the rodent affected ovarian follicular maturation and cyst formation. Several androgens have been used to induce an acute PCOS condition in rats through daily injection or subcutaneous implants, including dehydroepiandrosterone (DHEA), testosterone propionate (TP), and 5a-dihydrotestoterone (DHT). It is important to note that there is some inconsistency between studies in the reporting of endocrine hormones and ovarian histology in different models. In addition, several studies have not assessed Cardiometabolic parameters, and effects of daily androgen injection and/or treatment on physiologic indices such as body weight, stress indicators, or food intake are not usually reported. In these rodent models the pathological induction of PCOS is transient and dependent on androgen treatment. Hence the re-establishment of normal reproductive/ovarian cycling occurs after cessation of andro- gen administration.

DHEA-induced PCOS[13]: Dehydroepiandrosterone is the first androgen to rise in the female peripubertal period. It has been demonstrated that nearly 50% of follicular-synthesized T can be derived from circulating DHEA, and 25% of patients with PCOS demonstrate supranormal circulating DHEA concentrations. Dehydroepiandrosterone was first used by Roy et al. to induce PCOS in rats. Typically, prepubertal rats, aged approximately 22 days, are injected daily with DHEA (6 mg/100 g body weight, dissolved in 0.2 mL of sesame oil) for up to 20–27 days. After treatment, rats become acyclic and anovulatory.

Ovarian Morphology: Dehydroepiandrosterone-induced rats develop varied severity of cystic ovaries, with multiple follicular cysts ranging in size from 0.45 to 2.2 mm in diameter, and these have degeneration of granulose cell layers. The ovarian tunica capsule is not thickened, and the ovarian weight of DHEA-treated rats is increased significantly.

Endocrine hormone profile: Serum DHEA, T, E_2, FSH, LH, and PRL concentrations are significantly increased in DHEA-induced rats compared with control animals, whereas no changes in plasma FSH and LH concentrations have been reported by other groups.

Cardiometabolic abnormalities: Wang et al. showed that fasting serum glucose and insulin concentrations were in- creased in DHEA-induced rats. The resistin messenger RNA level of white adipose tissue was elevated, which may induce obesity-mediated insulin resistance (IR) in this model.

Summary: The DHEA-induced model can be used to reflect early DHEA-related hyperandrogenemia, anovulation and cystic ovaries, and development of aberrations in insulin/ glucose metabolism.

TP-induced PCOS[14]: Testosterone is used to induce polycystic ovaries in immature female rats. In this protocol, 21-day-old animals are injected daily with TP (1 mg/100 g body weight dissolved in propylene glycol) for up to 35 days.

Ovarian morphology: The TP-induced rats have large multiple cystic follicles, hyperthecosis, and a thickened tunica capsule. Corpora lutea fail to develop at 56 days of age, in contrast to control animals. The proportion of preantral follicles increases after TP treatmen, which may be associated with increased early follicular development, as observed in human PCOS.

Endocrine hormone profile: Serum T, LH, and PRL levels are increased, whereas FSH, P, and E_2 levels are decreased in TP- treated rats.

Cardiometabolic abnormalities: TP-treated rats have normal to decreased fasting glucose concentrations, and insulin was increased, leading to a signif- icantly reduced glucose/insulin ratio. The results of these studies suggest that it is possible that excess T may lead to hyperinsulinemia.

Summary: The TP model is a direct model of hyperandrogenemia, T-induced anovulation and cystic ovaries, and development of impaired insulin/glucose metabolism.

DHT-induced PCOS*(15):* DHT, a nonaromatizable androgen, can induce both ovarian and metabolic aberrations in rodents. Three-week-old rats (juvenile) are implanted subcutaneously with 90-day continuous-release pellets containing 7.5 mg DHT (daily dose, 83 mg). Plasma DHT concentrations are elevated 1.7-fold in treated compared with control animals. Irregular ovarian cyclicity and polycystic ovaries are evident after 11–13 weeks of the DHT treatment.

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Ovarian Morphology: Ovaries of DHT-treated rats display an increased incidence of atretic and cystic follicles, with reduced granulsa cell layers and hyperthecosis of internal cell layers. Interestingly, the ovarian weight of the DHT- treated animals is decreased, which is unlike other androgen-induced models and human PCOS (depending on the phenotype, for example not all cases present with enlarged ovaries).

Endocrine hormone profile: The plasma concentrations of T and E_2 are within the normal range, which is not representative of hyperandrogenemic phenotypes of PCOS, and P levels are diminished, indicating anovulation.

Cardiometabolic abnormalities: In addition to ovarian dysfunction, DHT-treated rats present with metabolic disturbances, including increased body weight associated with intra-abdominal adipose tissue, decreased insulin sensitivity (assessed by euglycemic– hyperinsulinemic clamp), and elevated plasma leptin concentrations. Despite these meta- bolic changes, which are consistent with PCOS and the metabolic syndrome in women, the DTH-induced model does not develop dyslipidemia. Approximately 70% of women with PCOS are dyslipidemia; therefore, the DHT-treated rodent model, which is resistant to blood lipid changes, may not be representative of this common phenotype of PCOS.

Summary: The DHT model mimics the actions of T and indicates androgen receptor–mediated effects. Although it does not demonstrate hyperandrogenemia, it does have irregular cyclicity and cystic ovaries, which may be used to represent the normoandrogenic phenotype of PCOS in women. The DHT-induced model also provides a model to explore the development of aberrations in insulin, glucose, leptin metabolism, and adiposity in PCOS.

Letrozole-Induced (Aromatase Inhibitor) Rodent Model of PCOS(15)

Aromatase is the key enzyme that converts T and androstenedione into E_2 and estrone, respectively. It is widely expressed in human tissues, such as placenta, ovary, and testis. Reduced aromatase activity in the ovary is one of the pathophysiologic hypotheses of PCOS development. Letrozole is a nonsteroidal aromatase inhibitor that reduces conversion of androgens to estrogens in the ovary, resulting in increased T and decreased E_2 production. Excess T in the ovaries is likely to cause polycystic ovaries directly in Letrozole-treated rats. The reduction in estrogen weakens the negative feedback on LH production in the pituitary, resulting in increased LH levels, which further stimulates theca cells to secrete T. Typically, 6-week-old female rats (puberty) are administered Letrozole orally at doses of 0.1, 0.5, and 1.0 mg/kg daily for 21 days, after which they become acyclic, with histological and biochemical features of human PCOS.

Ovarian morphology: The ovarian morphologic changes of Letrozole-induced rats include the development of cysts with hyperplasia of internal theca cells and a thickened ovarian capsule. The number of corpora lutea is decreased, indicating oligo/anovulation. However, unlike human PCOS, the ovarian weight of Letrozole-induced rats remains un- changed.

Endocrine hormone profile: The serum T and LH levels of Letrozole-induced rats are significantly elevated, and the P and E_2 levels are decreased in a dose-dependent manner. However, in this model, the FSH level is markedly increased in the higher-dose groups (0.5 and 1.0 mg/kg), which are not the typical characteristics of human PCOS.

Cardiometabolic abnormalitie: The metabolic characteristics of the Letrozole- induced rodent model of PCOS. After continuous administration of Letrozole (via a subcutaneous implant) before puberty (at 3 weeks of age) through to adulthood (12–16 weeks of age), no metabolic aberrations were observed, inclusive of adiposity, insulin sensitivity, and dyslipidemia.

Summary: The Letrozole model targets the study of aromatase deficiency–induced classic PCOS and may be an effective co treatment with other treatments that induce Cardiometabolic aberrations to study these factors in the PCOS condition.

HERBAL REMEDIES FOR PCOS

Panax ginseng (Ginseng): The roots of Panax ginseng (Araliaceae) is an herbal medicine. It is used as a tonic and it slow down the ageing properties. Ginseng saponins is active constituent of ginseng. They are composed of ginsenosides namely Rb1, Rb2, Rc, Rd, Re, Ro, Ra and minor ginsenosides. It is a suitable dietary supplement. Induced the polycystic ovary in rats by estradiol valerate. The study analyzed the ovarian morphology. Kampo preparations, is the one the ginseng containing formulation. It is formulation significantly decreases the plasma LH levels and thereby it is effective in improving endocrine condition in the treatment of disturbances of ovulation in patients with PCOS[16].

Tribulus terrestris (Puncture vine): Tribulus terrestris, (Zygophyllaceae) commonly known as Puncture vine or Devil's eyelashes plays an important role in traditional medicine. Tribulus terrestris was found to be effective in polycystic ovarian syndrome. In an investigation done in rats with polycystic ovaries induced with estradiol valerate, found that Tribulus terrestris extract is effective in improvement of ovulation in rats. The extract treatment normalized estrous cyclicity and steroidal hormonal levels and regularized ovarian follicular growth. Many herbalists find tribulus is an effective, overall ovarian stimulant and female fertility tonic, making it an excellent choice for women with polycystic ovary[17].

Gymnema sylvestre (Gymnema): Gymnema sylvestre (Asclepiadaceae) is a herb which is used in traditionally in Ayurvedic system of medicine. It has various pharmacological effects like antidiabetic, hypoglycemic, and lipid lowering effects. The active constituent of gymnema is saponins, especially gymnemic acids. gymnema has potential hypoglycemic activity in experimental models of diabetes. It regulates the blood glucose level. Metformin therapy for treatment of PCOS is convential. Therefore gymnema can be used for the underlying factor of insulin resistance. Gymnema is well indicated for PCOS, due to its insulin modulating activity and the added benefits of reducing the elevated triglycerides associated with PCOS.

Punica granatum (Pomegranate): Pomegranate (Punica granatum of the family Punicaceae) is one of the fruit and has various numbers of medicinal properties. The fruit contains folic acid, vitamins (B2, C, B1), sugars, pantothenic acid, and organic acids. The seed is reported to contain unsaturated and saturated fatty acids. The effect of

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pomegranate extract in the control or managment of PCOS was performed in adult female rats using control and PCOS group. The concentration of free testosterone, serum estrogen and andrestandoin hormone levels in experimental group was monitored. The study suggests the protective effect of pomegranate extract on hormonal imbalances of polycystic ovarian syndrome. The phenolic compounds and phytosterols found in the extract have positive effect in improving the complications of PCOS. The study recommends that the consumption of the extract reduces complications associated with PCOS[18].

Aloe barbadensis (Aloe): Aloe barbadensis Mill. (Liliaceae) popularly known as Aloe vera is a well-known plant with various medical properties and pharmacological activities. For management and prevention of polycystic ovarian syndrome the Aloe Vera gel has used. The biochemical clinical characters of PCOS were investigated using female rat model. The phytocomponents of aloe vera formulation were analyzed for flavonoids, polyphenols, sterols and other nutrients. The female rats were then treated orally with the Aloe vera gel formulation. This restored their glucose sensitivity, estrus cyclicity, and the enzyme activity. The histological analysis found, that aloe vera reduce the ovary ateretic cysts. The results were compared with PCOS control. The studies indicate that aloe vera has potential efficacy or beneficial effect in the prevention and maintenance of PCOS[8].

Cinnamomum zeylanicum (Cinnamon): Cinnamon (Cinnamomum zeylanicum of the family Lauraceae) has insulin potentiating properties. Cinnamon is reported to contain polyphenols and procyanidins. This compound regulates the insulin stimulated glucose uptake and glycogen synthesis. A pilot study conducted in fifteen women with PCOS and then fasting and oral glucose tolerance test values were measured. The cinnamon extract improved the insulin sensitivity in women with PCOS. The polyphenols and procyanidins found in cinnamon extract are responsible for the hypoglycemic effect by potentiating the insulin signaling pathway. The study established the role of cinnamon as an adjunctive therapy in the treatment of PCOS. Another study reported the effect of cinnamon on menstrual cyclicity and metabolic dysfunction in women with PCOS. It was a randomized controlled trial with 45 women. Cinnamon supplement were given orally. luteal phase progesterone level and menstrual cyclicity were monitored. The cinnamon supplementation improved the menstrual cyclicity and it is effective for poly cystic ovary syndrome[19].

Glycyrrhiza glabra (liquorice): Liquorice (Glycyrrhiza glabra of the family Leguminosae) has been used in traditional medicine to treat various of diseases. It has antifungal, antiviral, antibacterial, and antihyperglycemic properties. Glycyrrhizic acid is the important bioactive compounds in liquorice. Liquiritigenin, liquiritin, isoliquiritin, isoliquiritigenin, glabidin, glabrene are some of the phytoestrogens present in liquoirice. Reported effects on vascular tissues in vitro and in vivo of two natural compounds derived from liquorice root: glabridin, the major glabrene and isoflavan, an isoflavene, both demonstrated estrogen-like activities. Liquiritigenin a selective estrogen receptor ligand might be one of the bioactive compound responsible for weight loss. Other compounds glabrene and glabridin have showed the effect on weight reduction in vivo. It has also been reported that the combined treatment with spironolactone and liquorice in hirsute women is effective in PCOS, in order to reduce the volume depletion induced by spiranolactone and possibly enhance its anti-androgenic activity.[20]

Symplocos racemosa (Lodh Tree): Symplocos racemosa Roxb. From the family Symplocaceae, is a widely used Ayurvedic remedy mainly for female disorders. It is also known as Lodhra and is used in Indian System of Medicine as single drug or in multi-component formulation and preparations. The anti-androgenic properties S. racemosa in the treatment of PCOS was investigated in Letrozole induced female rat model. Syplocus racemosa treatment show significant recovery of estrogen, testosterone, progesterone levels and ovarian tissues. It is prevents ovarian cell dysfunction in PCOS and improved the fertility[4].

Linum usittatissimum (Flaxseed): Flaxseed is obtained from Linum usittatissimum (Linaceae) a food generally renowned for its omega-3 fatty acid content, also one of the richest sources of dietary lignan. Several biologically active compounds like alpha- linolenic acid (ALA), lignans (secoisolariciresinol diglycoside-SDG), soluble flaxseed fibre mucilage (d-Xylose, L-Galactose, LRhamnose, dgalacturonic acid) which have significant health benefits. The studies on the use of flaxseed or isolated lignan suggest that it may decrease androgen levels and normalize lipid levels. Lignans seem to reduce the excess testosterone which plays a key role in the pathogenesis of PCOS. A case study reported that flaxseed supplementation may indeed help regulate androgen levels in women with PCOS. A significant decrease in androgen levels was observed in the study. Decrease in hirsutism also observed. Findings suggest that flaxseed may have a profound impact on testosterone levels, and also may reduces symptoms associated with hyperandrogenism, such as hirsutism. Another study reported the effect of flax seeds on ovarian morphology in PCOS and it has showed that flax seed supplementation significantly reduced the ovarian volume, number of follicles in the ovaries and improved the frequency of menstrual cycles. However the study did not find any change in hirsutism, blood sugar level and body weight[21].

Curcuma longa (Turmeric): Curcumin is a water-insoluble, low molecular weight, polyphenolic curcuminoid derivative found in rhizomes of Indian spice, Curcuma longa of the family Zingiberaceae (turmeric). Turmeric is extensively used as a food additive and coloring agent in Asian cuisine and also in Indian herbal medicine. Curcumin has been reported to possess a wide variety of biological effects like anti-inflammatory, antioxidant hypoglycemic antihyperlipidemic activities and estrogenic effects. A study was conducted in 30 female Albino Wistar rats, using Letrozole-aromatase inhibitor, to induce Polycystic Ovarian Syndrome. Its effect was comparable to that of Clomiphene citrate, most widely used treatment for ovulation induction in PCOS condition. Serum levels of Progesterone and Estradiol were decreased in PCOS induced group. Curcumin restored the hormone and lipid profile, antioxidant and glycemic status as well as ovarian morphology in Letrozole induced PCOS animals. Decreased progesterone levels are also indicative of anovulation and curcumin successfully restore the ovulation. The study suggests that the effects may be attributed to its multiple pharmacological activities like

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estrogenic, antihyperlipidemic, antioxidant and hypoglycemic effects which could be useful in managing PCOS condition and prevent ovarian cell dysfunction, ovulation and thereby improving fertility. The studies show that the effect of curcumin is similar to that of Clomiphene citrate[22].

Cocus nucifera (Coconut): The reported the effect of Cocus nucifera (Arecaceae) flowers in reducing the major multiple symptoms of Letrozole-induced PCOD in female rats. Antioxidant status (superoxide dismutase (SOD) and glutathione reductase (GSH)) of the uterus homogenate, lipid profile (total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), and triglycerides (TG)) of the serum was determined. Weights of the uterus and ovaries were separately monitored. The characteristics of changes in the ovary were evaluated by histopathological studies. C. nucifera flower extract-treated groups showed increased uterus weight and estrus cyclicity which indicates the estrogenic effect. The improved ideal lipid profile, good antioxidant status, blood sugar level and histopathology results revealed the recovery from poly cystic ovaries. Histological findings of the treated groups indicated that the extract of C. nucifera may bring down the active levels of hormones, such as FSH and LH, to normal levels, and that may be the reason for the recovery from experimentally induced polycystic ovaries. This is further supported by the presence of methyl (9Z, 12Z)-9, 12-octadecadienoate which possesses anti-androgenic properties], in the GC-MS analysis of the extract. The GC-MS analysis of the aqueous alcoholic extract showed the presence of twenty-five volatile and semi volatile phytoconstituents. The presence of flavanoid (3, 5Dihydroxy-6-methyl-2, 3-dihydro-4Hpyran- 4-one), which is responsible for its hypoglycemic effects[16].

CONCLUSION

Polycystic ovarian syndrome (PCOS) is one of the common endocrine disorder in women of reproductive age. Various risk factors have been investigated in relation to PCOS, which including glucose intolerances, obesity and dyslipidemia. Although so many synthetic drugs are shown effective management, their number of side effects and high cost lead a way to seek plant based remedies for the treatment of PCOS. In this review summarize some important medicinal plants for the treatment of PCOS. It is medicinal plant help for improving and managing PCOD condition. These plants are improving hyperandrogenism, insulin sensitivity, improving fertility and improving menstrual cyclicity.

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A Review on Antidepressant Activity

Prajkta Phadtare*[1], Priyanka Ghadage[2], Abhirup Sagare[3], Rupali Bhoite[4], Karishma Yadav[5]

Students[1, 4, 5], Assistant Professor[2, 3]

Department of Pharmacology

Yashoda Technical Campus, Wadhe, Satara, India

Corresponding Author's Email id: *Phadtare.praj97@gmail.com[1*]*

Abstract

Depression is a widespread psychiatric disorder affecting around 21% population of the world. It is fourth leading cause of disease trouble universal by ranked and it is expected to turn into the second most immobilizing disorder. Moreover, it is not easy to expect which patient will retort to whichever given treatment. At present obtainable antidepressant drugs are effective and harmless, but limitations range from a delayed start of action to a considerable rate of non-responders. In the systems of traditional medicine, numerous plants and formulations have been used to take care of depression for thousands of years. The presently using drugs can impose a variety of side effects including cardiac toxicity, hypopiesia, sexual dysfunction, body weight gain and sleep disorder. During the last decade, there is a growing interest in the therapeutic effects of natural products on mental disorders.

***Keywords**: - Depression, Neurotransmitters, Antidepressant drugs, Mechanism, Pathophysiology, Medicinal plants*

INTRODUCTION

Depression is a chronic mental disorder that causes changes in mood, thoughts, behavior and physical health. It's a common but serious disease that can take away a person's ability to enjoy life and cause decline in capacity to undertake even the simplest daily tasks (1). Other than its chronic nature, symptoms associated with this mental disorder are often recurring and life threatening. According to the World Health Organization (WHO) unipolar depression is one of the leading causes of disability-adjusted life year (DALY) and approximately 350 people worldwide are

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said to suffer from this mental disorder (2). As described in the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM- V) , the hallmark of major depressive disorder (MDD) is the occurrence of depressed mood (dysphoria) and loss of interest in activities that were rather pleasurable in the past (anhedonia) for a duration of at least two weeks (3). These symptoms must also be accompanied by at least four of the following manifestations such as changes in appetite or weight, sleep patterns, altered psychomotor activity, feelings of worthlessness or guilt, difficulty concentrating or making decisions and recurrent thoughts of death or suicidal ideation (4). Even though there are plenty of drugs developed for the management of depression, one of the challenges in dealing with this disease is that a significant portion of the patients taking antidepressants fail to attain full remission (5). Some patients also develop treatment resistant depression in which the patients fail to respond to the available drugs or other therapeutic approaches.

DEPRESSION

Definition

Depression is a chronic mental disorder that causes changes in mood, thoughts, behavior, and physical health (6). It's a serious but common disease that cans away a person's ability to enjoy life and cause decline in capacity to undertake even the simplest daily tasks (7).

Symptoms

1. Feelings of unhappiness or sadness
2. Loss of pleasure in normal activities or interest
3. Frustration or irritability
4. Excessive sleeping or insomnia
5. Changes in appetite
6. Reduced sex drive
7. Restlessness or agitation
8. Slowed speaking, thinking or body movements
9. Tiredness, loss of energy, fatigue
10. Frequent thoughts of death, suicide or dying

Types of depression

1. Major depressive disorder

Major depressive disorder is also known as unipolar depression. In this type of disorder of depression typically show anhedonia and dysphoric mood followed by physical changes in such as enhanced or reduced appetite, weight gain or loss, sleep alteration in pattern and sustained fatigue (8). Disturb in executive and cognitive functions are also demonstration by coherent thinking and lack of

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concentrations as well as morbid brooding by thoughts of suicide and death (9).

2. Dysthymic Disorder

Dysthymic disorder is known as persistent depressive disorder (10). Patients display sadness or depressed mood that persists for the majority of the duration of the day for a minimum of two years in one year in adolescents and adolescents and adult. Majority of the major depressive disorder patients do not meet the full criteria of as there is interruption by remission of shorts periods (11).

3. Melancholic Depression

There is an almost absolute lack of ability to experience pleasure in all or almost everything. Morning mood is a worst and apparent in the psychomotor retardation is in this patients of subset (12). This type of depression is seen major frequently in the aged, in patient with psychotic depression and more severe form of depression (13).

4. Seasonal Affective Disorder

Seasonal affective disorder is depression type that's related to changes in seasons. This type of depression described as recrudesces by the year early winter or during fall (14). This seasonal affective disorder is characterized by feelings of guilt, low mood, increased irritability and worthlessness, shared symptom with other depressive disorder (15). Patients show a significant carving for foods high in carbohydrates and increased in appetite which results in obesity (weight gain).

5. Post-Partum Depression

Post partum depression is described group of heterogeneous depressive symptoms that have an effect on mothers (16). These symptoms may outward after or before giving birth. Half of the "postpartum" episode starts before the delivery time. Thus, are assigned to inclusively as "peri-partum" episodes (17). According to DSM-V anxiety and mood swings symptoms during pregnancy.

6. Psychotic Depression

Psychotic depression is depressive disorder is describe which is accompanied and very severe and psychotic symptoms. It is seen commonly as a combination of depression and psychosis that is not distinct into more of the two (18). It includes the symptoms of psychotic features such as delusions or hallucinations. Other than that its severe psychotic depression is related with poor response, prolonged course to higher relapse rate and available drugs (19).

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EPIDEMIOLOGY OF DEPRESSION

Depression is a large universal burden of disease and affects people in all communities across the world and 450 million people experience from several type of behavioral and mental disorder (20). The one year currency of depression is 4-8% and the lifetime currency for major depression as 14-17%. The lifetime currency rates of depression among men are 5-12% and women are 10-25%.

Moreover, bipolar disorder and major depression were associated with three fold increased risk of premature mortality as compared to general population.

The prevalence of depression in children is low and then increases in all the way through adolescence with a one-year prevalence of 4-5% in intermediate to late juvenescence (21). Depression is large risk aspect for suicide observed in adolescents; it's one of the main causes of death in this age group (22). Depression also leads to educational impairments and serious social and associated with substance abuse, increased rate of smoking, obesity.

ANTIDEPRESSANT DRUG

1) ***Selective serotonin re-uptake inhibitors (SSRIs)***
   e.g - Fluoxetine
   Citalopram

2) ***Serotonin and norepinephrine re-uptake inhibitors (SNRIs)***
   e.g - Amoxapine
   Desipramine

3) ***Monoamine oxidase inhibitors (MAOIs)***
   e.g - Clorglyline

4) ***Tricyclic antidepressants (TCAs)***
   e.g - Imipramine

5) ***Tetracyclic antidepressants***
   e.g - Amoxapine
   Desipramine

6) ***Serotonin receptor modulators (SRMs)***
   e.g - Trazodone

ANTIDEPRESSANT MECHANISMS

Neurotransmitters are endogenic chemicals that communicate signals covering a synapse from one neuron to another 'target' neuron. Brain neurotransmitters competency not be secreted in sufficient amounts to relieve disorders of mood (23). The chemicals like melatonin, dopamine and serotonin are the most essential in brain for sense. Onetime the nerves are robbed of those neurotransmitters, they

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can't send messages to different nerves which leads to depression (24). The messages that are passed direct the neurons are exhibited as behavior, emotions, appetite, temperature, or several alternative functions. Low levels of norepinephrine and serotonin within the conjugation area leads to depression (25). Hence, antidepressant like medicine used to treat this works by enhancing the number of restricted neurotransmitter in that specific part of the brain which authorizes to transfer the message (26).

All type of antidepressant works at brain among small differences, all antidepressant medicine affect the neurotransmitters how to work within the brain, mainly norepinephrine and serotonin, thus manage the balance of the neurotransmitters.

Selective serotonin reuptake inhibitors have different mechanism of action. SSRI has three different serotonin reuptake inhibitors those are paroxetine, sertraline and fluoxetine (27). These have selective effect for both fluvoxamine and citalopram on the serotonin reuptake pump. It leads to primary enhance in serotonin at the cell body and dendrites (28). So, SSRLs act by blocking the serotonin reuptake pump (5-HTT).

Serotonin norepinephrine reuptake inhibitors SNRIs block both norepinephrine transporter (NET) and 5-HTT. Blocking those transporters prohibit the neuron from vaccuming up extreme neurotransmitters, granting a lot of to stick in the synapse and excite postsynaptic receptors (29).

Monoamine oxidase inhibitors (MAOIs) MAOIs aren't retake blockers as all they enhance neurotransmitter amount by inhibiting monoamine oxidase (MAO), relate enzyme that breaks down all 3 monoamine like dopamine, serotonin, and monoamine neurotransmitter (30). Thus, MAOIs enhance the level of all 3 neurotransmitters thought critical in depression; these are adequacy advantage by others (31).

Tetracyclic and Tricyclic antidepressant alleviate depression by affecting naturally happen chemical messengers (32). Cyclic antidepressants generally block the results of 2 neurotransmitters known as norepinephrine and serotonin these are accessible in the brain. This looks to assist brain cells receive and send messages (33). The roles of these chemicals have treat the depression.

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Serotonin receptor modulators are used in the treatment of irritable intestine syndrome. Serotonin plays a important role in the humour reflexes and initiation of peristaltic, and in alteration of visceral sensations.

Lithium utilized for manic depression. Manic depressive patient's ability serious mood changes, starting from frenzied state to sadness or depression relate degree excited.

NEUROTRANSMITTER SYSTEM

The adrenaline, noradrenaline, dopamine, catecholamine from the adrenergic system in the Central Nervous System. Some of these adrenergic neurons discharge catecholamines in to the frontal cortex and radiate from the ancient limbic system (emotional centres) (2). The catecholaminergic pathways are responsible for mood stress (flight or fight), alertness responses. Serotonin is the main neurotransmitter regulates the excitatory catecholamine system of the Central Nervous System. Serotonin neurons are important as the control of mood, appetite, sex drive, memory (34).

The noradrenaline and serotonin system have their most important cell bodies in small region of the brain stem that serve as headquarters for sending axonal projections throughout the brain in specific pathways that mediate specific functions (35). Multiple noradrenergic and serotonergic pathways may be dysfunctional in depression generating many different symptoms.

The projection of the serotonin system arises from the nuclei of the raphe magnus and dorsal raphe. The serotonin receptors (5-HT) have been identified into various sub-types with 5-HT1 and 5-HT2 sub types being of greater interest in psychiatry. Subclass is 5-HT1A which is concentrated in hippocampus and raphe. These receptors are implicated as autoreceptor that modulate 5-HT release from presynaptic neurons. The 5-HT2 receptor occurs in high concentration in the nucleus accumbens and frontal cortex.

HYPOTHESIS OF DEPRESSION

Several hypotheses of the biological determinants of depression have emerged over the most important of these and the implications therefore are reviewed below. Today it is generally accepted that depression is not necessarily due to a shortage of one vital brain neurotransmitter, but rather to a disruption in the equilibrium between different regulatory systems

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1. The monoaminergic hypothesis of depression

This hypothesis has develop to contemplate the prospect that depression can be the result of a inadequacy in signal transduction against the monoamine neurotransmitter to its postsynaptic neuron, even with normal levels of receptor and neurotransmitter being present (3). The monoaminergic hypothesis of depression accept that the major symptoms of depression are the result of inadequate concentration of serotonin (5-HT) and noradrenaline (NA) in the synaptic clefts related to neurons in the brain (36). Emerging theories that link environmental and genetic and risk factors for depression by resulting in less key gene products, down regulating certain genes, such as brain derived neurotrophic factor (BNDF), being produced. So if the encoding gene is repressed the result may be atrophy or even apoptosis of neurons (37).

2. The dopamine hypothesis of depression

The original hypothesis was formulated in the late nineteen seventies by Solomon snyder and linked schizophrenia with dopamine (DA) activity (38). Later this hypothesis was extended to include depression following the observation that many antidepressants influence the metabolism of dopamine following chronic antidepressant treatment, the presynaptic DA receptors become subsensitised and this result in an increase in DA release (39). A decrease in homovanllinic acid (HVA), the main metabolite of dopamine, in the cerebral spinal fluid (CSF) of decreased patients who demonstrate marked motor retardation has also been reported (40). Therefore, a reduced in the ratio of HVA to DA is indicative of decreased turnover of DA (41). This hypothesis is also supported by reports of significantly reduced dopamine turnover in depressed suicide victims (42).

3. The permissive hypothesis of depression

This hypothesis emphasizes 5HT as a neuromodulator and its importance as a target for antidepressant action. According to this theory, a lowered concentration within the Central Nervous System (CNS) of 5-HT results in an affective state regulated by NA (43). Decreased NA and 5-HT levels will give rise to depression. This means that 5-HT can act as a permissive modulator of neurotransmitter functions through connections between serotonergic pathways and make connections with dopaminergic and

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noradrenergic pathways via the associated receptors (44).

4. ***The glutamatergic N-methyl –D-aspartate hypothesis***

Current discovery specify that the affliction of CNS glutamatergic pathways can play a role as in mechanism involved in depression (45). Several studies have independently confirmed that compounds which reduce activity at the NMDA receptors produce similar effects to clinically active antidepressants. It is therefore hypothesized that adaptive changes in the NMDA receptor complex could be a common pathway affected by all antidepressants (46).

MEDICINAL PLANTS USED IN DEPRESSION

Medicinal plants throughout the world have been utilized to treat disorders of the body and the brain since antiquity. Herbal medicine has been a proper alternative for the management of mental disorder such as depression, anxiety, and dementia between plenty others. Discovering antidepressants from herbal sources appear to be proper approach due to their lower prevalence of side effects and therapeutic efficacy (47). Hyperforin and hypericin are flavonoids present in hypericum that are claimed to be important for the antidepressant activity of the plant.

Medicinal plants largest universally used to treatment of depression throught the world are *Centella asiatica, Hypericum perforatum, Rauwolfia serpentina, Pfaffia paniculata, Schizandra chin, Rhododendron molle, Thea sinensis, Valeriana officinalis, Uncaria tome, Withania somnifera.*

There is a long history of using plants for treating different diseases in Ethiopia. This herbal based therapy is high valued and has passed from one generation to another generation by word of mouth. Herbal therapy closed continues to be the first preference treatment option for nearly 80% of the population. Plants such as *Whitiana somnifera, Justica odora, Calpurnia aurea and Aspargus leptocladodius* have traditionally been used for depression treatment (48).

1. ***Clitoria ternatea***

Mood disorders are one of the major common mental sicknesses with a lifetime risk of 10% in the widespread population. Most of the drugs that are currently being used within the treatment of depression adversely influence the quality of life of the patients. This leads to patient's non-

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compliance with medication, which further complicates the problem.

In the present study, clitoria ternatea (150 and 300 mg/kg) produced significant dose dependent antidepressant effect in behavioral despair tests. The plant clitoria ternatea contains tannin; the antidepressant activity may be due to MAO inhibition, thereby enhancing norepinephrine and dopamine levels in the brain (11).

2. Zingiber officinale

Ayurveda mentions a number of single and compound drug formulations of plant origin that are used within the treatment of psychiatric disorders and are claimed to have a better side-effect profile than conventional drugs.

Zingiber officinale (150 and 300 mg/kg) significantly ($p<0.001$) and dose dependently decreased the immobility time as compared to group of control mice. Thus, the activity of zingiber officinale could involve particular mechanisms of the established agents as described above.

As medicinal plants possess their importance considering ancient time, people are using it from different ways by medicine source. From the highly beneficial animal study, we achieve that the plant extract zingiber officinale show a significant antidepressant activity in TST and FST models of depression (20).

3. Magnolia officinalis

Magnolia officinalis, M. dealbata, M. grandiflora, M. obovata and are the plants from family Magnoliacea which are used to treatment of neurological diseases such as depression, convulsion, seizure, and anxiety and as sedative and painkiller.

Magnolol and honokiol are two main compounds identified in these plants. These compounds came to be reported to cause antidepressant effects through affecting serotonergic system investigated the effect of the oral use of such two compounds at mild chronic stress induced depression. Mild chronic stress caused reduced in 5-HT and its metabolite, 5-HIAA, in different parts of the brain and abolish the activity of platelets adenylyl cyclase.

These two compounds caused the changed value of adenylyl cyclase, 5-HIAA, 5-HT, and corticosterone to return to baseline levels. The antidepressant effects of honokiol and magnolol in this study were assign to the improvement of the induced disturbance in HPA axis, AC-cAMP pathway, serotonergic system (49).

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4. Hypericum perforatum

In the current years, H. perforatum has exist competing for being commercially accessible as an antidepressant and for this reason some studies have been manage to discover the chemical compounds responsible for this effect and their action mechanisms. Biochemicals investigations possess determine that H. perforatum is a weak inhibitor of monoamine oxidase but inhibits synaptosomal restoration of dopamine, serotonin, and norepinephrine. H. perforatum extract exerts down-regulatory effect on beta-adrenergic receptors and up-regulatory result on serotonin receptors, and changes the neurotransmitters concentrations in definite regions of the brain.

The antidepressant effects of H. perforatum certain compounds like as Hyperforin, hypericin, and isoquercetin have been demonstrated (50).

5. Rosmarinus officinalis L.

R. officinalis is from family Labiatae and has numerous pharmacological effects including ant diabetic, antibacterial, and anti-oxidant, hepatoprotective, anticoagulant, antiulcer, diuretic and anti-inflammatory. An experimental study showed that hydroalcoholic R. officinalis extract (100 mg/kg) with treatment significantly reduced immobility duration in suspension in mice and forced swim test. Pretreatment with pchlorophenylalanine (serotonin synthesis inhibitor), NAN-190 (receptor antagonist 5-HT1A), ketanserin (receptor antagonist 5-HT2A), mCPBG (antagonist receptor 5-HT3), prazosin (1 adrenoreceptor antagonist), SCH23390 (D1 dopamine receptor antagonist), and sulpiride (D2 dopamine receptor antagonist) inhibited the antidepressant effects of R. officinalis extract (49).

6. Passiflora foetida

At the basis of the clinical association of stressful life events and depressive episodes, many of the animal models for the antidepressant evaluation of drug activity evaluate stress –precipitated behaviors. Harmaline alkaloids present in p.foetida doing by reversible monoamine oxidase inhibitors and in common with other beta caroline binds to HT (5-hydroxy tryptamine) receptors. MAO regulates the metabolic degradation of catecholamines, serotonin and other endogenous amine in central nervous system. Inhibition of this enzyme causes a reduction in metabolism and subsequent increase in the concentration of biogenic amines. Also the flavonoid components of MERF might be interacting with adrenergic and

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serotonergic systems in mediating the antidepressant effects of MERF (51).

CONCLUSION

Depression is an incapacitating disease which needs appropriate treatment. This presentation reviews the pharmacology of antidepressant drugs and the future perspectives of treating mood disorders such as depression. The foremost theory for explaining the biological basis of depression has been the monoamine hypothesis. Depression is due to a deficiency in one or other biogenic monoamines (serotonin, 5-HT; noradrenaline, NA; dopamine, DA). Antidepressant drugs are therefore classified according to their ability to improve monoaminergic transmission. Since this first theory, other explanations based on abnormal function of monoamine receptors or associated with impaired signaling pathways have been suggested. Notable progress has been accomplished in the treatment of major depressive disorders with new compounds recently discovered (selective serotonin reuptake inhibitors: SSRI; serotonin noradrenaline reuptake inhibitors: SNRI). Behavioral, electrophysiological and micro dialysis studies have shown that serotonin (5-HT) receptors exert a key role in modulating antidepressant activity.

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Review on Antiulcer Activity of Different Herbal Medicines

Rupali Jadhav[*], Karishma Yadav[1], Prajkta Phadtare[2], Amruta Tidke[3], Aryan[4]
Students
Department of Pharmacology
Yashoda Technical Campus, Wadhe, Satara, India
Corresponding Author's Email id: *rupalipbhoite@gmail.com*

Abstract

Peptic ulcer disease is one of the common gastrointestinal disorders. The conventional drugs used in the treatment of ulcers include histamine receptor antagonists, prostaglandin analogues, proton pump inhibitors, cytoprotective agents, antacids and anticholinergics. Hence, herbal medicines are generally used in such chronic cases, wherein drugs are required to be used for long periods. A number of screening methods are used for evaluating antiulcer activity, such as pylorus ligation in rats, Stress ulcer through immobilization stress, Stress ulcers by cold water immersion, Indomethacin induced ulcers in rats and ethanol-induced mucosal damage in rats. In this review number of herbal remedies has mentioned as they have minimum side effects and a greater margin of safety.

***Keywords**: - Prostaglandin analogues, Proton pump inhibitors, Zollinger-Ellison syndrome, Ulcer index, Immobilization stress, Cytoprotectives etc.*

INTRODUCTION

Peptic ulcer disease is a serious GI disorder. These ulcers can develop when the imbalance occurs between the gastro-protectives (mucus, bicarbonate and prostaglandins) and aggressive (acid, pepsin, bile salt and Helicobacter pylori bacteria). Here are major factors that cause peptic ulcers like infection with gram-negative Helicobacter pylori, increased hydrochloric acid secretion, inadequate mucosal defense against gastric acid, drugs, such as cholinergic drugs, NSAIDs, improper food intake. Peptic ulcer disease is one of the common gastrointestinal disorders in clinical practice. The common forms of peptic ulcer are duodenal ulcer, gastric ulcer; NSAIDs induced ulcer and

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stress ulcer. Among these, the duodenal ulcer is more common in adult males. A gastric ulcer occurs commonly at old age and a lower socio-economic class of individuals. The current approach to the peptic ulcer is managed by inhibition of gastric acid secretion, blocking apoptosis, promotion of gastro-protection, and stimulation of epithelial cell proliferation for effective healing. The conventional drugs used in the treatment of ulcers include histamine receptor antagonists, prostaglandin analogues, proton pump inhibitors, cytoprotective agents, antacids and anticholinergics, but most of these drugs produce undesirable side effects or drug interactions and may even alter biochemical mechanisms of the body upon chronic uses. Hence, herbal medicines are generally used in such chronic cases, wherein drugs are required to be used for long periods. (Hamilton & Rege, 2004), (Milosavljevic, Kostić-Milosavljević, Jovanović, & Krstić, 2011)

CHANGES IN LIFESTYLE AND DIETARY

Aspirin and related drugs (non-steroidal anti-inflammatory drugs), alcohol, coffee (even decaf) and tea can interfere with the curing of the peptic ulcers. Smoking may also low the ulcer healing process. People with ulcer symptoms have been evaluated to have more carbohydrates than people with no ulcers, from this route may occur with a genetic susceptibility for the ulcer pathogenesis. Sugar has also been reported to increase stomach pH. Salt may cause stomach and intestine irritation. Large uptakes of salt have been linked to a higher risk of stomach ulcers. One of the amino acids known as Glutamine is the important energy source in cells that cover the stomach and intestine. It also prevents the stress ulcer related by large burns of the preliminary study about the pathogenesis of ulcers. (Fichna, 2017)

TYPES OF PEPTIC ULCER

1) Gastric ulcer
2) Duodenal ulcer

Gastric Ulcer

Gastric ulcers are usually single and less than 20 millimetre in diameters. Ulcers on the small curvature are mainly related to the chronic gastritis condition, whereas those in the larger curvature are often associated to the non-steroidal anti-inflammatory drugs effects Physiological factors in gastric ulcers: Gastric ulcers almost invariably arise in the setting of H. pylori gastritis or chemical gastritis that results in injury to the epithelium. Most patients with gastric ulcers secrete less

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acid than do those with duodenal ulcers and even less than normal persons.

The factors implicated include:

1) back-diffusion of acid into the mucosa,
2) Decreased parietal cell mass,
3) Abnormalities of the parietal cells themselves.

A minority of patients with gastric ulcers exhibit acid hypersecretion. In these persons, the ulcers are usually near the pylorus and are considered variants of duodenal ulcers. Interestingly, the intense gastric hypersecretion that occurs in the Zollinger-Ellison syndrome is associated with severe ulceration of the duodenum and even the jejunum but rarely with gastric ulcers. (نواس ال, 2016)

Duodenal Ulcer

Duodenal ulcers are ordinarily located on the walls of the duodenum, at a short distance from the pylorus region. Physiological factors in duodenal ulcers: The maximal capacity for acid production by the stomach reflects total parietal cell mass. Both parietal cell mass and maximal acid secretion are increased up to twofold in patients with duodenal ulcers. However, there is a large overlap with normal values and only one-third of these patients secrete excess acid. Accelerated gastric emptying, a condition that might lead to excessive acidification of the duodenum, has been noted in patients with duodenal ulcers. However, as with other factors, there is substantial overlap with normal rates. Normally, acidification of the duodenal bulb inhibits further gastric emptying. The pH of the duodenal bulb reflects the balance between the delivery of gastric juice and its neutralization by biliary, pancreatic and duodenal secretions. The production of duodenal ulcers requires an acidic pH in the bulb, that is, an excess of acid over neutralizing secretions. (BOCK & KELLEY, 1949)

Symptoms of Peptic Ulcers

- Changes in appetite
- Nausea
- Bloody or dark stools (melena)
- Unexplained weight loss
- Indigestion
- Vomiting

SCREENING METHODS FOR ANTIULCER ACTIVITY

1) Pylorus Ligation in Rat (SHAY rat)

This model is a simple and convenient method for the induction of gastric ulceration in the rat through ligature in the pylorus region; the ulceration is affected by the accumulation of acidic juice inside

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the stomach. Ulcer index & pH of gastric content of treated animals are compared with control groups. Different cumulative group administration, followed by dose-response curves establishment for ulcer formation, can be measured in these methods. Procedure: Female Wistar rats weighing 150-170 g are starved for 48 hours having access to drinking water ad libitum. During this time, they are housed singly in cages to prevent coprophagy. Six animals are used per dose and as control groups. Under mild ether anaesthesia an incision is made at the abdominal midline. The pylorus is closed by using small nylon. Higher supervision is required to avoid the damage of blood vessels inside the pylorus region. Grasping the stomach with instruments is to be meticulously avoided; else, ulceration will invariably develop at such points. The abdominal walls are sutured through the surgical procedure. The test samples are administrated through oral ingestion or injected subcutaneous route. The animals are placed for 19 hours in a suitable plastic container. Afterward, these animals are sacrificed in CO2 anaesthesia. The abdomen is re-ligated and a ligature is placed above the esophagus region and closer to the diaphragm area. The stomach is replaced with a watch glass and the materials are collected into a centrifugal tube. Above the longer curvature, the stomach fully opened and pinned between cork plates. The mucosal layer is observed with the help of a stereomicroscope. (Bae et al., 2011)

2) Stress Ulcer through Immobilization Stress

Psychogenic factors, such as stress, produce a major role in the etiology of gastric ulcers in human beings and animals. Hence not only antacids ingestion, anticholinergics, H2–antagonists, proton pump inhibitors treatment, and also psychotropic agents such as neuroleptics have also effective for the treatment.

Procedure: Groups of 6 female Wister rats per dose of test drug and for controls weighing 150-170 g are used. Food and water are removed 24 hours before the experimental procedure. After oral or subcutaneous ingestion of the test substance or the placebo drug in animal's extremities is fixed combine and the animals are tied in wire gauze. These animals are horizontally suspended in a dark room at 20oC for one day and the last animals are sacrificed in the CO2 anaesthesia method. The stomach has been cut, fixed in a cork plate and the count and scores the severity of ulcers with the help

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of video recorded stereo-microscope. (نواس ال, 2016)

3) Stress ulcers by Cold Water Immersion

Coldwater treatment of rats during the restraint duration boosts the appearance of gastric ulcers and reduces the time necessary immobilization process. Procedure: In this experiment, groups of 8-10 Wistar rats weighing 150-200 g were used. After oral administration of the test compound, the rats have placed vertically individual restraint cages in the water at 22oC for 1 hour. These were removed, allowed to dry and inject Evans blue (30mg/Kg) intravenously through the tail vein. After ten minutes, these animals are sacrificed by CO2 anesthesia; stomachs were collected in Formol - saline (2%v/v) overnight storage for about 24 hours. After that, the stomachs are opened the greater curvature, washed via warm water and examined through a 3-fold magnifier. (Ito, Shichijo, & Sekine, 1993), (Goulart et al., 2005)

4) Indomethacin Induced Ulcers in Rats

Nonsteroidal anti-inflammatory agents, like indomethacin and acetyl-salicylic acid, produce gastric lesions in human beings and rodents by the inhibition of gastric cyclo-oxygenase leading to the formation of prostacyclin. Procedure: Groups of 5-6 Wistar rats weighing 150 - 200 g are used. The test drugs are administered orally in 0.1 % tween 80 solutions 10 minutes before the oral indomethacin at a dose of about 20 mg/kg (4 mg/ml dissolved in 0.1 % tween 80 solutions). Six hours later, the rats are euthanatized through CO2 anaesthesia; these animals stomach was removed and inject with Formol-saline (2%v/v) for storage about one day. After that, the stomachs are opened the greater curvature, washed via warm water and examined through a 3-fold magnifier. (Bozkurt et al., 2017)

5) Ethanol Induced Mucosal Damage in Rats

(Cytoprotective activity) The intragastric application of absolute ethanol is a reproducible method to produce gastric lesions in experimental animals. The lesions may be blocked by some drugs (ex:- prostaglandins). These protective activity opposites to the irritants are known as a cytoprotective activity. Procedure: Male Wistar rats weighing 250 - 300 g are deprived of food 18 hours prior to the experiment but are allowed free access to water. During this time, they are kept in restraining cages to prevent

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coprophagy. The rats are administered either are appropriate vehicle or cytoprotective drugs, for example, a prostanoid, intragastrically 30 minutes prior to administration of 1 ml absolute ethanol. Untreated animals are included as controls. One hour after administration of ethanol, the animals are sacrificed in CO2 anaesthesia and their stomachs exercised, cut along the greater curvature and gently rinsed under tap water. The stomachs are stretched on a piece of foam core mat, mucosal site up. The subjective scores of the treated tissues are recorded.(Sahoo et al., 2016)

NATURAL REMEDIES

Bari Ilayachi (Elettaria cardamomum and Amomum subulatum)

Both drugs have a gastroprotective effect may be due to a decrease in gastric motility. They cause relaxation of circular muscles, which may protect gastric mucosa through flattening of the folds. This will increase the mucosal area exposed to narcotizing agents and release the volume of gastric agents on the rugal crest. Such action has been postulated to play a role in the cytoprotective effect of prostaglandins. (Jamal, Javed, Aslam, & Jafri, 2006)

Black berry

One of the most interesting substances that have been obtained from chili peppers and present in spicy plants such as ginger or black pepper is capsaicin. This substance acts on sensory neurons to stimulate their membrane receptors, principally vanilloid (VR)-1 receptors, and free various kinins such as substance P. When applied in a large dose; capsaicin destroys selectively C-fiber neuronal endings leading to inactivation of sensory nerves and the loss of all reflexes in which these nerves are involved. In a lesser dose, capsaicin is a potent gastroprotective agent and a stimulant of gastric microcirculation. (Sangiovanni et al., 2013)

Chamomile (Matricaria recutita)

Chamomile is a herb that has been used traditionally as a mild sedative to relieve anxiety and in treating digestive disorders, including peptic ulcers. Chamomile also may be effective in relieving inflamed or irritated mucous membranes of the digestive tract and in promoting digestion. Chamomile has a soothing action on the digestive system. Its gentle, soothing action is beneficial in digestive disorders like indigestion, acidity and peptic ulcers. It is also rich in the flavonoid apigenin-another flavonoid that inhibits the growth of H. pylori. (Jabri et al., 2017)

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Dong quai (Angelica sinensis)

Animal studies recommend that dong Quai may relieve ulcers, but studies in people are needed before a definitive conclusion can be drawn. (Ye, So, Liu, Shin & Cho, 2003)

Licorice (Glycyrrhiza glabra)

Licorice root has a high background in the effect of soothing effect in the inflamed and damaged mucous membranes of the digestive tract. Licorice also protects the stomach and intestinal parts by increased production of mucin, which protects the lining of the HCl and other substances. According to Preclinical research, Flavonoids of licorice may also suppress the growth of H. Pylori.(Jamal et al., 2006)

Marshmallow Root (Althaea officinalis)

For decades, marshmallow has been used in folk medicine to help cure gastric ulcers. The roots of the marshmallow contain mucilage, a gelatinous substance found in plants. When it comes into contact with water, this mucilage swells, forming a soft, protective gel. This is believed to provide a protective barrier against irritating substances that may aggravate ulcers. (Zaghlool, Shehata, Abo-Seif, & Abd El-Latif, 2015)

Tea Root Extract (Camellia sinensis)

Tea root extract might primarily decrease the leakage of plasma proteins into the gastric juice with the strengthening of the mucosal barrier and increase its resistance to the damaging effect of ethanol-induced ulcer.(Maity, Vedasiromoni, & Ganguly, 1995)

Turmeric (Curcuma longa)

Some of the important constituents of Curcuma longa exert several protective effects on the gastrointestinal tract. Sodium curcumin, which is a salt of curcumin, inhibits intestinal spasm, and p-tolymethylcarbinol was found to be capable of increasing bicarbonate, gastrin, secretin, as well as pancreatic enzyme secretion. Turmeric also inhibits ulcer formation caused by stress, alcohol, indomethacin, pyloric ligation, and reserpine in rats. This study demonstrated turmeric extract significantly increased the gastric wall mucus in rats subjected to these gastrointestinal insults.(Rafatullah, Tariq, Al-Yahya, Mossa, & Ageel, 1990)

Antiulcer Activities of Bambusa Arundinacea

The anti-inflammatory activity shown by the methanol extract of Bambusa arundinacea is due to prostaglandins synthesis inhibition. One such possibility

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is that the anti-inflammatory and antiulcer activity of the methanol extract may be due to the inhibition of PAF receptors. Significant anti-inflammatory activity was also noticed with the methanol extract on immunologically induced inflammation. The methanol extract also showed the significant antiulcer activity.(Muniappan & Sundararaj, 2006)

Anti-ulcer Activity in a Hydro- ethanolic Extract from Kielmeyera coriacea

To induce gastric ulcers in experimental animals the antiulcer activity of a hydro-ethanolic extract prepared from the stems of Kielmeyera coriacea Mart.(Guttiferae) was evaluated by using different models like ethanol-acid, acute stress and Indomethacin models. Treatment with K coriacea hydro-ethanolic extract provided considerable antiulcer protection in the ethanol-acid and Indomethacin models however it did not treat the acute stress model. These results concluded that the K coriacea hydro-ethanolic extract protect the gastric mucosa by increasing resistance to necrotizing agents. (Goulart et al., 2005)

Gastric Antiulcer and Anti-Inflammatory Activities of the Essentialoil from Casearia Sylvestris Sw

To explore the antiulcer and anti-inflammatory activities of the essential oil from Casearia sylvestris leaves (EOCS) the following tests were used: pylorus ligation test, rat paw edema, granulomatous tissue test, vascular permeability, writhing test, gastric ulcer stress-induced. The overall total yield of EOCS was 2.5% having a LD50 of 1100 mg/kg in mouse. Caryophyllene, thujopsene, alfa-humulene, beta-acoradiene, germacrene-d, bicyclogermacrene, calamenene, germacrene B, spathulenol and globulol are the major compounds identified using gas chromatography. The EOCS orally administered to the rats at 125 mg/kg given out 36% of inhibition in carrageenan-induced edema in the rat paw assay.($p < 0.05$, Student’s t-test). However, both rat paw edema dextran-induced and vascular permeability assayusing histamine showed no significant inhibition. Mice submitted to the writhing test using acetic acid presented 58% and 56% of inhibitionin writhes with EOCS and indomethacin, respectively. Furthermore, EOCS inhibited 90% of stress-induced gastric ulcer, while cimetidine inhibited 70% ($p < 0.05$, Student’s t-test). The volume of gastric secretion in the group treated with EOCS was greater than the group treated with cimetidine. With this it can be concluded that EOCS of Casearia sylvestris possess

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anti-inflammatory and anti-ulcer activity. (Esteves et al., 2005)

CONCLUSION

A peptic ulcer is one of the serious gastrointestinal tract disorders characterized by severe damage to epithelial cells of the stomach characterized by GI bleeding. The number of allopathic medicines used nowadays is usually accompanied by side effects like nausea, vomiting, constipation, indigestion, heartburn, blood in vomiting etc. Hence, herbal medicines are generally used in such chronic cases; drugs are required to be used for long periods. Bari ilayachi, blackberry, curcuma amada, marshmallow root, liquorice root, curcuma longa are some of the herbal remedies that possess very good cytoprotective properties, increases bicarbonate ion and prostaglandin production and can be used for a long period of time.

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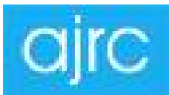

REVIEW ARTICLE

Insilico Molecular docking analysis in Maestro Software

Avanti K. Galande*, Sachin H. Rohane
Department of Pharmacy, Yashoda Technical Campus, Satara- 415015, India.
*Corresponding Author E-mail: **avantigalande7938@gmail.com**

ABSTRACT:
The maestro is the scientific leader in developing state-of-the-art chemical stimulation software for use in pharmaceutical, biotechnology, and, materials science research. Maestro is the portal to all of maestros computational technology-far more than just a user interface, Maestro also helps researches organize and analyz data. Maestro is the culmination of years of research and development. by working closely to be the user environment that is both intuitive and allows our users to get work done efficiently. The typical bacteria form a biofilm which barrier for antibiotics and other harmful substances. The capacity of phages to penetrate that. In the study, we showed that Galloflavin and Ellagic acid increased SIRT6 activity and decreased the expression of SIRT6 associated proteins involved in cancer development. Taken together, Galloflavin and Ellagic acid targeting SIRT6 activity may provide a new insight in the development of anti-cancer therapy. As cetuximab exhibits several anticancer mechanisms, in vivo studies are needed to explore and confirm the effects of combining osimertinib with cetuximab in the L858R/T790M/L792H-mutant pattern.

KEYWORDS: Molecular docking, drug discovery, Maestro software.

1. INTRODUCTION:
Computer-aided drug design uses computational approaches to discover, develop, and analyze drugs and similar biologically active molecules. The ligand-based computer-aided drug discovery (LB-CADD) approach involves the analysis of ligands known to interact with a target of interest. These methods use a set of reference structures collected from compounds known to interact with a target of interest and analyze their 2D or 3D structures. The basic objective of these methods is to predict the nature and strength of binding of given molecule a target. The program renders the rover in a 3D environment. The program features the jet Propulsion Laboratory testing facility. Spirit's landing site to explore. Data from the Spirit and Opportunity's landing site must be downloaded externally from the Maestro website and imported into the program [1-3].

REVIEW OF LITERATURE:
Wieslaw swietnicki and et al used Maestro software to check the in silico analysis of bacteriophage tail tubular suggests a putative sugar binding site and a catalytic mechanism. In silico analysis was performed on the structure of a base tailplate protein gp31 from *Klebsiella pneumonia* bacteriophage KP32 (PDB: 5MU4) which shows activity towards maltose but not trehalose. The first region clearly favored maltose during the docking phase while the second one allowed for the energetically-equivalent binding of trehalose [4].

Carmen Diez-Simon and et al used Maestro software to check the comparison of volatile trapping techniques for the comprehensive analysis of food flavourings by gas chromatography-mass spectrometry. Trapping volatiles is a convenient way to study aroma compounds but it is important to determine which volatile trapping method is most comprehensive in extracting the most relevant aroma components when investigating complex food products. Comprehensiveness and repeatability were compared and SBSE proved particularly suitable for extracting components such as polysulfides, pyrazines and terpene alcohols [5].

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Jae Myung Park and et al used Maestro software to check the A dodecapeptide selected by phage display as a potential theranostic probe for colon cancers. The peptide probe maintained binding affinity even after serum incubation. For therapeutic applications, this peptide probe was conjugated to hematoporphyrin, a photosensitizer, which showed a significantly enhanced cellular uptake and high photodynamic effect to kill tumor cells [6].

K. Wan Yusof and et al used Maestro software to check the developing a UiTM (Perlis) web-based of building space management system: A preliminary study in locating a specified space/room area using open source GIS tool. The preliminary result of the study shows that the spaces and room areas in the building can be mapped out digitaly and it can also be made available to be accessed through the web for the resident of the university [7].

Iman Mirmazloum and et al used Maestro software to check the oxidative stress level and dehydrin gene expression pattern differentiate two contrasting cucumber F1 hybrids under high fertigation treatment. According to RT-qPCR transcript levels of several antioxidant enzymes genes (ascorbate peroxidase, glytathione reductase and glutathione peroxidase) were significantly higher in 'Joker' compared to 'Oitol'. Antioxidant capacity increased in both hybrids with strong characteristics differences favoring 'Oital' plants [8].

Minna Rahnasto-Rilla and et al used Maestro software to check the effects of galloflavin and ellagic acid on sirtuin 6 and its anti-tumorigenic activities. Ellagic acid increased the deacetylase activity of SIRT6 by up to 50-fold; it showed moderate inhibition of SIRT1-3. Galloflavin and ellagic acid showed anti-proliferative effects in Caco2. In this study, we showed that Galloflavin and Ellagic acid increased SIRT6 activity and decreased the expression of SIRT6 associated proteins involved in cancer development. Taken together, Galloflavin and Ellagic acid targeting SIRT6 activity may provide a new insight in the development ofanti-cancer therapy [9].

Susan M. Burden-Gulley and et al used Maestro software to check the A novel molecural diagnostic of glioblastomas: Detection of an extracellular fragment of protein tyrosine phosphate μ[1.2]. The activity of the receptors tyrosine kinase is normally kept in check by the opposing activity of RPTPs such as PTPμ, which are important regulstors of adhesion-dependent signals [10].
H. Bounouria and et al used Maestro software to check the study of heavy metal assessment in the gharb plain along sebou river (morocco) using k0-NAA method triga mark II research reactor. The Ko-mothod activation analysis (ko-NAA) was used in order to determine the concentration of major and trace elements in sediment samples collected from different sites in the Gharb plain aong the Sebou River (morocco). The comparison with other sussequent studies on Sebou River gives an idea about the temporal evolution of heavy metal contamination at national scale [11].

Prasad G. Jamkhande and et al used Maestro software to check the software based approaches for drug designing and development: A systemtic review on commonly used software and its applications. Drug Discovery includes drug designing and development, is a multifarious and expensive endeavor, where least number of drugs that pass the clinical trials makes it to market. Novel software based methods such as molecular modeling; structure-based drug design, structure-based virtual screening, ligand interaction and molecular dynamics are considered to be powerful tool for investigation of pharmacokinetics and pharmacodyanamic properties of drug, and structural activity relationship between ligand and its target [12].

Sejal P. Gandhi and et al used Maestro software to check the computational data og phytoconstituents from *Hibicus rosa-sinensis* on various anti-obesity targets. Molecur docking analysis of twenty two phytoconstitient from *rosa-sinensis*, against seven targets of obesity like pancreatic, hormones as ghrelin, leptin and protein as SCH1and MCH is detailed in this data article. Chemical structures of phytoconstituents were downloaded from PubChem[2][13].

Sabrin R.M. Ibrahim and et al used Maestro software to check the mangostanaxanthone VIIII, a new xanthone from *Garcinia mangostana* pericaps, α-amylase inhibitory activity, and molecular docking studies. The α-amylase inhibitory potential of the isolated metabolites was evaluated. The molecular docking study of the tested metabolites was estimated to shade up therational explanation of the α-amylaseinhibitory activity results [14].

Amin O. Elzupir and et al used Maestro software to check the inhibition of SARS-CoV-2 main protease $3CL^{pro}$ by means of α-ketoamideand pyridine-containing pharmaceuticals usingin silico molecular docking. This study report for the firsttime a compound that could be binding to ALA^{285}, the new residue reaulting from genetic modification of $3CL^{pro}$ of SARS-CoV-2 that hasincresed its catalytic activity 3, 6-fold compare with its predecessor $3CL^{pro}$ of SARA-CoV [15].

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El-Sayed I. El-Desoky and et al Maestro software to check the synthesis, biological evaluation and *in silico* molecular docking of novel 1-hydroxy-naphthyl substituted heterocycles. Chalcone **3c**, naphtyl pyrazoline **6b** and hyadroxycoumarin **13** exhibited the higher activity as antioxidants. *In silico* molecular docking of pyrazoline **6b** endorsed its proper binding at the active site of the 2EX6 enzyme which explains its potent antibacterial activity in comparison with standard ampicillin [16].

Raveendra Melanvanki and et al used Maestro software to check the investigation of interaction between boronic acids and sugar: effect of structural change of sugars on binding affinity using steady state and time resolved fluorecencespectroscopy and molecular docking. Binding interactions of boronic acid derivatives viz. 2-Methylphenylboronic acid (B1) and 3-Methoxyphenylboronic acid (B2) withmono saccharides (arabinose, fructose and galactose) and (sucrose, lactose and maltose) in aqueous condition at pH 7.4 by means of fluorescence spectroscopy is reported in the present investigation [17].

Massound Amanlou and et al used Maestro software to check the anti-HCV anti-malaria agent, potential candidates to repurpose for coronavirus infection: Virtual screening, molecular docking, and molecular dynamics simulation study. Concurrent the viral entranceto the host cell, its antigen will exposure to antigen presentation cells (APC) and then identified by cytotoxic T lymphocytes (CTIs). But reducing the number of CD4and CD8 cells in COVID-19 patients prevents Tcell proliferation and activity [18].

Mohammed M. Matin and et al used Maestro software to check the novel mannopyranoside esters as sterol 14α-demethylase inhibitors: synthesis, PASS prediction, molecular docking, and pharmacokinetic studies. Te activity spectra analysis along with *in vitro* antimicrobial evaluation clearly indicated that those novel MDM estershad better antifungal activitites over antibacterial agents [19].

Andrea Barni and et al used Maestro software to check the Mini-factories for close-to-customer manufacturing of customized furniture: from concept to real demo. During demonstration, customers had the possibility to access the shop, configure their products and see them manufactured in quasi-real time. The promising results of the demonstration activity pave the way for further exploration of the proposed concept [20].

Shobana Sundar and et al used Maestro software to check the Molecular docking, molecular dynamics and MM/PBSA studies of FDA approved drugs for protein kinase a of Mycobacterium tuberculosis; application insights of drug repurposing. Tuberculosis (TB) is a deadly disease, and novel treatment strategies are required to combat it. Repurposing of existing Food and Drug Administration (FDA) approved drugs against Mycobacterium tuberculosis (Mtb) proteinscould be beneficial for TB treatment [21].

CONCLUSION:

After completion of review, it was found that the Meostro molecular docking softwear is very important for Insilico analysis of different pharmaceutical compounds, it aslo helps in determine the molecular interaction, Molecular binding.

This softwear also useful to check the pharmacological activity, therapeutic activities such as Anti tumorigenic activity, Anti HIV activity, Anti malarial activity ETC. After literature review it was seen that numbers of researchers suggest the Meostro softer used for molecular binding, molecular interaction and insilico analytical activity.

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REVIEW ARTICLE

Review on Guassion, the General Purpose in Computational Chemistry for Medicinal Chemistry

Shubham A. Nangare*, Sachin H Rohane
Department of B Pharmacy at Yashoda Technical Campus, Satara.
*Corresponding Author E-mail: **shubhamnangare251@gmail.com**

ABSTRACT:
In these review we explain all the detailed information about guassian software. Now a days the guassian very much beneficial in to computational chemistry for medicinal chemistry work by the various calculations. This is initially used bye the john poples. Guassiansoftware capable of predicting many properties and calculations of molecules and reaction. Molecular docking also done bye this software. Varios authors wordks on their subject by using this software. I shows interest into guassian because of this is very beneficial for calculations. In guassian varios mathematical equations are added and this will be feneficial or helpful to guide scientist.

KEYWORDS: Molecular docking, Drug Discovery, Guassion Software.

1. INTRODUCTION:

Guassian software is a general purpose computational chemistry software package. Guassian software initially started used in or relesed in 1970 by the scientist john pople. And the scientist john pople started his research group at the Carnegie Mellon University as guassian 70 then this continusly udtaed by them. the name of software originates from scientist pople's use of guassian orbitals to speed up the molecular electronics structure calculation opposed to using slater type of orbital then choice to 9 improve the the performace of the software on computating capacities of current computer hardware for hartee fock calculations. The current updated version of this is guassian 16. This is originally availble through quantum chemistry programme exchnage. it was later licensed out by the university carnegie mellon university ans since 1987 has been developed and licensed by the guassian.

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Guassian:

Original author	**John poples**
Developers	Carnegie mellon university
Initial release	1970, 50 years
Stable release	Guassian 16/2017
Website	Www.guassian.com

We will use the guassian programme in windows environment. Guassian is capable of predicting many properties of molecules and reaction, including the following

- Molecular energies and structures
- Reaction pathway
- NMR properties
- Energies and structures of transition states
- Bond and reaction energies
- Vibrational frequecies
- Molecular orbital
- Atomic charges and electrostatic potential
- Multiple moments

Computation can be carried out on system in gas phase and in their ground state or in an excited state

Guassuian input files:

In this Guassian input files inclufdes several different sections.

- Link 0 commands- locate and name scratch files we will not use this option
- Route section- specify desired calculation type the

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method basic sets and other options
- optional addition sections- additional input needed fo9r specific job type
- Title section- brief description of calculation.

JOB TYPES FOR GUASSIAN INPUT:
There are 3 key components to this specification
1. Job type
2. The method
3. Basic set.

Computer aided drug design:
Simply rational design is the inventive process of finding new medication based on biological target. The drugs are commonly organic small moleculesthats activates or inhibits function of biomolecules such like a protein. That is further give the therapeutic action to the patient. Basic in that isdrug design means the invole in molecules that complementry in shape and size of biomolecules. They bind with each other and form the bond. Drug design not relies on computer modelling. This type of modelling callled as computet aided drug design. Drug design depends on knowledge of 3D structures of biomolecules that is known as structural aided drug design. In addition to small molecules biopharmaceutical includes peptides ans especially therapeutic antibodies are incresingly important class of drug and computational method for improving affinity, selectivity and stability been developed.

Drug design also known as efforts to develop a new drug by molecular modification of lead compound for optimization of desired efferts and minimization of side effects.

Now a days structural based drug design is the growing, interative and powerful approaches includes the structural evaluation of target and drug discovery process it is time consuming and as well as cost cunsuming too developing ideas of new effects and potential drug lead molecule

MOLECULAR DOCKING:
Molecular docking is very useful and intresting beneficial to us docking means the attempt to find best matching between two molecules. Docking is the process in which prdedict the preffere orientation of one ligand when bound in an active site to form stable complex. Aim for the molecular docking is to achive an optimization conformation for both receptor and ligand and the relative orientation between protein and ligand such that free energy of overall system is minimized. successful dockingvmethod search high dimensional spaces effectively and use a scoring function that correctly ranks candidate docking. Importance of the molecular docking is that identification of the ligands, correct binding geometry, prediction of binding affinity. etc. therae are rigid docking is the part of molecular docking in that we studied about internal geometry of receptor and ligand. Another type of docking is flexible docking in that we studied about the enumaration on rotation of the one of the molecules is performed. There are various application of molecular docking like lower free energy structures, calculate differntial binding of ligands, library design, novo design, screening of side effects, specificity of potential drug etc [1-3].

REVIEW OF LITRETURE:
Molecular studies docking charge transfer excitation and wave function analyses valacuclovir a potential antiviral drug this study carried by author Fathima Rizwana and Christina susan abraham and software used is guassian 0.9 [4].

Quantum chemical insight into molecular structure NBO anlysis of hydrogen bonded interaction spectroscopic drg likeness and molecular docking of novel anti covid 19 author for this is SJ. Jenepha Mary and C james study carried by the software guassian 0.9 [5].

Conformational anlysis and quantum descriotors of 2 new imidazole derivative by experimental DFT, AIM molecular docking studies adsorption activity on ghraphene study by author Veena S kumar and MS roxy software used by them is guassian 0.9 [6].

Computational assesment on wave function anlysis molecular conformation and molecular docking explores on 2-5 amino-2methylanilino-4-3 pyridile pyrimidine study by author K arulabraham and S mutha and software is guassian 0.9 [7].

Quantum computational spectroscopic and molecular dockinnn studies on 2acetylthoprene and its bromination deerivatives author is M habib rahman and M raja software is guassian [8].

Synthesis of 1-2-3 bistriazole derivative of embaline and evaluation of its effect on high fat diet streptozotacin induced type 2 diabetes in rats and molecular docking author for this antony stalin and perumol palani guassian 0.9 software used by them [9].

Studies of charged transfer complex og qunodic acid with carboxyllic acid, R kavitha and Biological evaluation mol docking and DFT rajendran are the authors and guassian is software used by them. [10].

Conformational profile vibrational abign NLO properties and molecular docking of biological active herbicide 1,1 dimethyl phenyleurea K haruna and al Saudi software guassian is used 0.9 [11].

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Detailed quantum mechanical, mol docking QSAR prediction, photovoltaic light homesting efficasy analysis of benzil and its halogenated analogus author Yshyama mary B suresh kumar guassian software used for this. [12].

M abhinaya and etal are the authors for the inhibition of biofilm formation quarnum sensing activity wae done on isolated 3-5-7 tri hydroxy and lave from alstonia scholoris lead by using chemistru guassian 0.9 [13].

New thiazide pyridine and pyrazole derivatives as antioxidant bcandidates synthesis DFT calculations and molecular docking by using guassian softwaew by eatal and yassine kaddouri [14].

2D QSAR and docking study of series gaumerin derivatives as inhibitions of CDR with an applicatiomof molecular docking bu guassian software author is Ranina kasmi and etal [15].

Y shyama and etal do study on the DFT and molecular docking investigation of oxicum derivatives was studied by using guassian [16].

Nasima arshad and etal studied on the structural elucidation DNA binding DFT molecular docking and cytotoxic activity studieson novel design crystal thiosemicarbazides was studied by using guassian software [17].

K haruna and etal bdo study on the confirmational profile and the vibrational assignments NLO properties and molecular docking of biological active herticides 1-1 dimethyl 3- phenykurea studies by guassian software [18].

Y shyamo mary and etal do study on the detailes quantum mechanical molecular docking QSAR prediction photovoltic light havsting efficasy analysis of benzil and its halogenated analogus studies by using guassian 0.9. [19].

Mohammad abdul mumit and tarum kumar studied on DFT studies on vibrational and electron spectro homo lumo, MEP HOMA, NBO and molecular docking analysis of benzyl, hydrazine carbodition by using guassian [20].

Towaeds better modelling drug loading in solid lipid nanoparticles molecular docking experiments done by author Rania hathout, abdelkader a metwally by using guassain software [21].

Spectral investigation, DFT computation and molecular docking studies of the Antimicrobial 5 nitroisatin dimer VK rastogi and VB joyhy are the authors, guassain software used by them. [22].

Investigation of DNA RNA molecules for efficasy and activity of corrosion inhibition by DFT and molecular docking tuzun and C kaya are authors. [23].

CONCLUSION:

The review totally focused on prediction of many properties of molecules and reactions using this software. Guassian software is an computer program helped to chemists, chemical engineers, physicist, biochemist and other scientist to predicting many properties of molecules and reactions. such as energies molecular structures, spectroscopic data ie NMR IR UV etc. These prediction based on present review have been becoming a helpful tool to guide scientist for the prediction of various properties.

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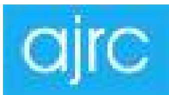

REVIEW ARTICLE

Review on Discovery Studio: An important Tool for Molecular Docking

Shravani S. Pawar*, Sachin H. Rohane
Department of Pharmacy, Yashoda Technical Campus, Wadhe, Satara 415003.
*Corresponding Author E-mail: **shravanipawar118@gmail.com**

ABSTRACT:
In this paper, an overview on discovery studio docking program for analyze and modeling molecular structure, sequence of relevance to life science researcher. This software covers the areas such as ligand design, structure-based design, macromolecule design and engineering, simulations, pharmacophore modeling, quantitative structure activity relationship (QSAR), ADME, predictive toxicity. Discovery Studio help to analyze activities such as anti-convulsant, anti-viral, antidepressant, antibacterial, anti-tubercular, anti-Alzheimer, anti-malarial, anti-cancer. This software gives better result correlation with in-view pharmacological activity. So, this is a helping tool for a researcher to minimize the time as well as cost of research activity and also gives better understanding to study ligand and receptor complex.

KEYWORDS: Drug Discovery, Molecular Docking, Discovery Studio software.

INTRODUCTION:

Computer-Aided drug design represents computational resources that used to facilitate the design and discovery of new therapeutic solutions. Molecular docking is widely used in CADD due to its reliable prediction properties and is one of the essential backbones of CADD. Molecular docking is a rapid and inexpensive technique in academics as well as in industrial settings. Molecular Docking is a technique to find best matching molecular structure by interaction between enzyme and ligand. It also analyses the orientation of one molecule with other into the binding site of a macromolecular target. Docking has a major role in virtual screening, drug discovery, bioremediation.

Discovery studio software is an agglomeration to transcript small molecules and macromolecule system. It is developed by Dassault Systemes BIOVIA (Accelrys). Discovery Studio is a single unified, graphical interface for advanced drug design and protein modeling research. This software provides bunch of viewers for display plots and graphical representation of data.

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Review of literature:

Ana-Maria Udrea and et al use Discovery Studio software to check the potential of phenothiazine in Covid-19 infection. They found that data given by docking software is correlated with in vivo activity. The data found to be suitable and correct with in vivo activity.

Shiben Wang and et al synthesized the different series of 1,3,4-oxadiazole derivatives using discovery studio and the compound were studied gives best anticonvulsant activity. In silico studies were carried out to explore the binding interaction of the most active compound. They found the target compounds were related with in vivo and vitro activity.

Fatma Gur and et al studied the adverse effect of Atomoxetine which issubstitute for methylphenidate in the long-term treatment of ADHD. They conduct molecular docking study using Discovery Studio programs. The data found to be match with in vivo activity.

K. Sangeetha and et al use the discovery studio to check the antiviral activities of plant derived compounds against zika virus. By In silico studies, the software used for screening of various phytochemicals against Zika virus to identify new promising drug candidates. In this study, around 5550 phytochemicals retrieved from

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various databases were subjected for molecular docking in Discovery studio program.

Shi-Ben Wang and et al designed and synthesized the derivatives of coumarin and 3, 4-dihydroquinolinone. They found that compound check in the discovery studio software have best antidepressant activityand also exhibits good affinity for the 5-HT1A receptor. These findings can be useful in the design and synthesis of novel antidepressants.

Fathima Rizwana B and et al studied the molecular docking and binding structures on famciclovir and entecavir compound simulated from Discovery studio program. The geometric structures were optimizedand the band gap energies were calculated using software. Electrostatic Potential (ESP) maps identifies Negative and positive potential regions with help of software. Molecular docking studies confirmed the antiviral activity of the selected compounds.

Geethalakshmi Rajarathinam and et al uses discovery studio for docking of T. decandra with FabZ. They found that isolated flavonoid compound possess excellent anti-P. aeruginosa activity. The in-silico analysis of isolated compound shows possible action in a hypothetical way. The molecular docking of flavonoid was carried out using Discovery Studio.

Sugunadevi Sakkiah and et al developed 3D pharmacophore model based on the known inhibitors. This Pharmacophore model was generated using HYPOGEN algorithm in discovery studio program. From the molecular docking studies around 36 compounds were obtained based on consensus scoring function and selected as HSP90 inhibitors.

The aim of Ran Joo Choi and et al was to evaluate the anti-Alzheimer's disease activities of selected ginsenosides. They use the docking software to check thepotential of ginsenosides in the development of therapeutic agents for Alzheimer's disease. They predict binding energies of the ginsenosides with β-site amyloid and obtained result were corelated with in vitro activity.

Nafees Ahmed and et al use discovery studio for synthesis of tricyclic guanidine derivatives and biological evaluation against P. falciparum. The docking studies show that there is very strong correlation between in silico and in vitro results. Based on the data obtained by software, more potent inhibitor against P. falciparum can be designed. Docking was performed using DS program to understand the mechanism of inhibition and to identify pharmacophore required for anti-malarial activity.

Prashant Bhardwaj and et al uses DS program for identification of novel proteintargets for triclosan. They conduct the inverse virtual screening study for protein targets. A text mining study of triclosan was initially performed to find outinteraction in various biochemical processes.

Amer Hosny and et al use the software for development of a predictive model to identify potential HIV-1 attachment inhibitors. They performed the study in two phased computational process to identify useful compounds capable of binding to the protein for therapeutic purposes using the Discovery Studio docking and screening software.

Mohammad Heiat and et al conduct the study in docking program to analyze isolated ssDNA aptamers against angiotensin II. They found that the structural and sequential homology between aptamers can be considered as a sign of similar characteristics and Output PDB files were modified from RNA to DNA in the discovery studio visualizer software. The in-silico study was performed and uses to find aptameric fragments binding potency.

Sagir Yusuf Ismail and et al performed in-silico QSAR study of sulfur containing shikonin oxime derivatives. The docking study also carried out between this derivatives and target protein. This study provides an approach for the design of more potent anti-colon drug. The data found to be match and correct with anti-cancer agent for colon cancer.

Shola Elijah Adeniji and et al use discovery studio software to molecular docking evaluation of selected quinoline derivatives. Discovery Studio software was used to visualized and analyzed the docked results. They found that activity of quinoline derivatives checked in software exhibits as best anti-tubercular agents.

CONCLUSION:

This study paved better understanding of Discovery Studio software for viewing, sharing, analysing protein and small molecule data. The DS program provides applications covering areas including molecular mechanism, molecular dynamics, quantum mechanics. Software also have ability to perform hybrid QM/MM calculations. It employed for small molecule and macromolecule applications. The molecular properties can found by editing structures and performing calculations.

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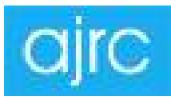

REVIEW ARTICLE

Efficiency of AUTODOCK: *Insilico* study of Pharmaceutical Drug Molecules

Uday M. Satpute*, Sachin H. Rohane
Department of Pharmacy, Yashoda Technical Campus, Satara - 415015, India.
*Corresponding Author E-mail: **udaysatpute650@gmail.com**

ABSTRACT:
In current situation lots of Molecular Docking Software are available in the market for *Insilico* study of pharmaceutical drug molecules, so that we have to choose most appropriate software. During literature survey it was found that AUTODOCK software was efficiently guided to as author's regarding mainly pharmacological activities such as Antidiabetic activity, Antimalarial activity, Antivirus activity, Anticancer activity, Anti mycobacterial tuberculosis activity, Antioxidant activity, Etc. After review it was analyse that the AUTODOCK and its Tools are more efficient to determine the synthesis techniques, spectral analysis, docking simulation, photochemical activities, therapeutic effects, toxicological studies.

KEYWORDS: Molecular docking, Drug discovery, Auto dock software.

INTRODUCTION:
In the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using, for example, scoring functionsThe associations between biologically relevant molecules such as proteins, peptides, nucleic acids, carbohydrates, and lipids play a central role in signal transduction. Furthermore, the relative orientation of the two interacting partners may affect the type of signal produced (e.g., agonism vs antagonism). Therefore, docking is useful for predicting both the strength and type of signal produced.

Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site. Characterisation of the binding behaviour plays an important role in rational design of drugs as well as to elucidate fundamental biochemical processes [1-3].

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REVIEW OF LITERATURE:
Sukanta Kumar Pradhanand et al used the Autodock 4 softwear for conducting several studies and identifing several target sites which influence drug-resistant to M. tb strains. In this case, there is the interaction between the protein Arabinosyl transferase C and two existing drugs (Ethambutol and Isoniazid) and by calculating the binding affinity and mode of binding. Ethambutol formed the five modified molecules (Emb 1, Emb 2, Emb 3, Emb 4 and Emb 5). The Emb 1 and Emb 3 having binding affinity- 5.77Kcal/mol and -5.13Kcal/mol respectively that are potential inhibitors of Arabinosyl trasferase C in mycobacterium tuberculosis [4].

Mohammed Al bratty used the Autodock Vina softwear for studying the extent and types of binding interaction present in between HAS and Anti Hypertensive drug like telmisartan (TLM). HAS is responsible for binding and transportation of many exogeneous and endogeneous ligand including drug like telmisartan in binding interaction, the TLM significantly interacts with binding site-1 of HAS by forming strong Hydrogen with Glu292 and Lys195 residues that affects concentration of TLM at site of action and also affects on therapeutic effect [5].

Idhayadhulla Akabar and et al used Auto Dock Vina softwear for the molecular interaction study between target protein and ligands. This study more exposed the all inhibitor acquired the negative dock energy against

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the target protein. In molecular docking investigation it was found that the natural coumarin analogue toddacoumaquinone shows the inhibitory activity with binding energy -7.8Kcal than other compound against main protease of SAR coronavirusin intricate with a-ketomide [6].

Ran Yu and et al used the AutoDockVina softwear in the current spread of noval coronavirus (SAR-CoV-2) situation to discover the Anti Viral drug. This softwear helps to screening the potential drug by molecular docking with structural protein and non-structuralprotein site of new coronavirus. The Ribavirin, remdesivir, chloroquine and luteolin are also studied, the luteoline is bind with high affinity to same site of the main protease of SARS – CoV-2 [7].

Fareeda Athar and et al used the AutoDock. 4.2. for molecular docking studies performed that all derivatives along with the standard inhibitor STX-0119 showed that binding energy released in direct binding with the SH2 domain of STAT3 was the highest for compound 5e (-9.91kcal/mol). In virtual screening, compound 5e was found to exhibit optimum competency in inhibiting STAT3 activity. Compound 5e decreased the activation of STAT3 as observed with Western blot. The compound 5e was identified as a potent antioxidant agent and STAT3 inhibitor and effective agent for cancer treatment [8].

Akinwunmi O. Adeoye and et al used AutoDock softer for Molecular docking and virtual screening to understand the mechanism of ligand binding and to identify potent calcium transporter inhibitors: This study also deals with the evaluation of inhibitory activity of secondary metabolites of ethylacetate partitioned-fraction of Adansoniadigitata stem bark extract on malaria-associated protein using in silico docking studies. Digitata stem bark extract were examined for their antiplasmodial activity. Digitata shows the binding energy ranging between -6.5kcal/mol and -7.1kcal/mol. Among the two chemical constituents, apigenin has the highest docking score along with the highest number of hydrogen bonds formed when compared to quercetin. The analysis results suggest that apigenin and quercetinare acts as an anti-malaria agent [9].

Mohammad Jakir Hosen and et al used the AutoDock Vina softwear for the screening of the drugs against RdRp of SARS-CoV-2. It has been found that RNA dependent RNA polymerase (RdRp) plays a crucial role in SARS-CoV-2 replication, and thus could be a potential drug target. This study revealed that Rifabutin, Rifapentine, Fidaxomicin, 7-methyl-guanosine-5′-triphosphate-5′-guanosine and Ivermectin have a potential inhibitory interaction with RdRp of SARS-CoV-2 and could be effective drugs for COVID-19 [10].

PrashamsaKoirala, Su HuiSeongand et al used the AutoDock 4.2 softwear for determined the molecular interaction of BACE1 with isolated terpenoids. The AutoDock 4.2 programme revealed that hydroxyl group of lupeol formed two hydrogen bonds with the ASP32 (catalytic aspartic residue) and SER35 residues of BACE1 with the binding energy of (−8.2kcal/mol), while the ketone group of lupenone did not form any hydrogen bonds with BACE1 giving evidence for less binding affinity. It predicted that the dependence of the inhibitory activity in the presence of hydroxyl group which has provided a new basis for BACE1 blockade [11].

A. Lakshmana Rao and et al used the AutoDock 4.2.6softwear. The docking procedure was applied on a set of designed ligands within the region of 2PRG active site using AutoDock 4.2.6 software. Based on the validations and hydrogen bond interactions of various substituents, they were considered for the evaluation. It was done to understand the kind of interactions that occurred between various substituted thiazolidine-2, 4-diones with 2PRG binding site region [12].

Lakshmana Rao ATMAKUR and et al used AutoDock softwear for In vitro anti-inflammatory activity was checked by human red blood cell (HRBC) membrane stabilization and protein denaturation. Using AutoDock, molecular docking studies were carried out to find out the best fit ligands. In molecular docking studies, compounds 4h and 4n exhibited higher binding affinity at PPARγ receptor protein and compound 4k exhibited higher binding affinity at COX-1 and COX-2 actives sites Compounds 4h and 4n exhibited higher binding affinity at PPARγ receptor protein and compound 4k exhibited higher binding affinity at COX isoenzymes' active sites in molecular docking studies. AutoDock 4.2.6 software was utilized to know the type of interactions of the designed 3D-structured thiazolidinediones with the 2PRG, 1EQG, and 1CX2 active site regions [13].

GanugapatiJayasreeand et al used AutoDock 4.0 for theDocking studies which are essential to understand the interaction between the protein and the ligands. In this case the crucial protein from Insuline receptor and ligands from Cinnamon. Docking studies are essential to understand the interaction between the protein and the ligands. In Autodock studies, the best pose was obtained with least energy value from which it can be hypothesized that these 2 compounds can be considered as potential activators of insulin receptor. After that the lab studies have to be performed to confirm the

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properties of these 2 compounds. tubulin isotypes. This indicates that select triple-modified 4 –chloro thio colchicines derivatives representhig hlypromising novel cancer chemotherapeutics. All the poses generated by both programs were rescored using the Vina scoring function. The top binding pose of colchicine and the derivatives was predicted by the Vina scoring function for the α-βII. a model which produce the best result sin terms of pIC50 predictions. Active residues involving non-hydrophobic interactions with the ligand are also specified for each compound [14].

Sarfaraz Alamand et al used AutoDockVina software (Scripps Research Chandrajit Dohutia and et al used AutoDock 4.2 to identify the receptor protein PfATP6 was the common target of artemisinin and curcumin. It was initiated to assess the antimalarial activity of six curcumin derivatives based on their binding affinities and correlating the in silico docking outcome with in vitro antimalarial screening. A ligand library of thirty two Knoevenagel condensates of curcumin were designed and docked against PfATP6 protein and six compounds with the best binding scores were synthesized and screened for their antimalarial activity against the sensitive 3D7 strain of Plasmodium falciparum [15].

Adam Huczynski and et al used AutoDock Vina and DOCK 6.5 for In silico studies to predict binding modes of the 4-chloro thiocolchicine derivatives to different β Institute, La Jolla, CA, USA) for molecular docking studies to validate the LibDock score. The designed compounds are optimized and then use for docking experiments. The docking program takes the PDBQT file format of ligands and receptor, a modified PDB file, which has added polar hydrogens and partial charges [16].

Leena K Pappachen and et al used AutoDock version 4.0 screeing program for doking the Benzothiazole derivatives with the crystallographic structures of the targets. AutoDock screening program also used to know about the hydrogen bonding interactions of all the derived compounds. The number of hydrogen bonding will considerably increase the affinity of ligand target interaction. The AutoDock shows that most of the benzothiazole derivatives show higher hydrogen bonding between the ligand-target interactions. The hydrogen bonding interactions increases the binding energy of ligand-protein interactions. The docking scores obtained for benzothiazole derivatives (BT1, BT2, BT3, BT4) and std. tamoxifen from the preliminary docking program using using AutoDock program were −6.29, −5.25, −7.19, −7.48, −3.86, r. All the four derivatives were synthesized, characterized, and subjected to in vitro anticancer screening by MTT assay in breast cancer (MCF-7) cell lines. Compounds DBT1, DBT2, and DBT3 were the most active compounds against MCF-7 cell lines with inhibitory concentration 50% of 70.0, 64.0 and 65.0, respectively. All the four derivatives show good docking scores when compared to standard drug tamoxifen and can be concluded that all the synthesized benzothiazole ligands show good anticancer property [17].

Gurudeeban Selvaraj and et al used AutoDock 4.0 for the docking analyses, There are different bonding modes of one ligand with multiple active cavities of DPP-IV. The docking analyses indicate that the bioactive constituents, β-stigmasterol, barbamide, docosahexaenoic acid, arachidonic acid, and harman shows the best binding energies on DPP-IV receptor and hydrogen bonding with ASP545, GLY741, TYR754, TYR666, ARG125, TYR547, SER630, and LYS554 residues. This result shows that docosahexaenoic acid, arachidonic acid, β-stigmasterol, barbamide, harman, ZINC58564986, ZINC56907325, ZINC69432950, ZINC69431828, ZINC73533041, ZINC84287073, ZINC69849395, and ZINC10508406 act as DPP-IV inhibitors [18].

J. Selvaraj and et al used the AutoDock softwear for validation through structure analysis verification server. For identification, the new potential drugs against GLUT4 protein the molecular docking studies of 20 natural compounds were carried out using AutoDock. The results shows that modeled structure has 87.9% residues at the core region.It was also shows that the good binding interactions of the ligand with both the targets at very low energy level. Based on the docking energy value, H-bond interaction the compounds hesperidin, fisetin, eriodictyol, wogonin, and chrysin was selected as the most potent compounds for GLUT4 protein. Hence, It was conclude that the compound shows the Anti Diabetic activity [19].

Natarajan Kiruthiga and et al used the AutoDock 4.2 for identify the binding modes of titled compounds responsible for the activity on the receptor sites. The compound HFd with 2, 4-dimethoxy group on ring C and 7-hydroxy substitution on ring A showed binding interactions with amino acid residues of alpha amylase as Arg 61, Pro 44, His 299, Gln 41 and Asp 96. Hence the scaffolds were acts as a navigator in the management of diabetic mellitus [20].

Saravanan R.R and et al used the AutoDock program for the docking simulations in the active sites of 2XNU, which shown the successfully reproduce binding modes in terms of lowest docking energy. The target protein structures of 2XNU were docked with MPIPA which shows the excellent results as were seen by the least values of the binding energy with the help of Autodock

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v4.0, In docking studies of the title derivative it understand that the possibility of these compounds to act as effective inhibitors [21].

Humaira Nadeem and et al used the AutoDock Vina softwear forThe interactions between the compounds and active site residues of H^+/K^+ ATPase. SCH28080 was used to validate the docking results. The results clearly indicate that these novel benzimidazole-pyrazole hybrids can present a new class of potential anti ulcer agents and can serve as new anti-ulcer drugs after further investigation [22].

Chandrajit Dohutia and et al used AutoDock 4.2 for receptor molecule for the docking and also study to probe the binding free energy between the ligand library and receptor. Autodock Tools (ADT) also used for optimize the receptor and ligand molecules. For preparation of the receptor molecule, polar hydrogens, Kollman charges and AD4 type of atoms were added, while Gasteiger charges were added on the ligands and maximum numbers of active torsions were given [23].

CONCLUSION:

This review totally focused on efficiency of AutoDock softwear and its different version such as AutoDock Vina, AutoDock 4.0, AutoDock 4.2.6 for molecular docking study. This information become benchmark for reseachers those uses the AutoDock softwear for study the different therapeutic activities like Antidiabetic activity, AntiMalarial activity, Antiurase activity, Anticancer activity, Antimycobacterial tuberculosis activity, Antioxidant activity, Etc. The AutoDock and its Tools are more efficient to determine the synthesis techniques, spectral analysis, docking simulation, photochemical activities, therapeutic effects, toxicological studies. These observation based on the present review have been becoming a helpful tool to guide researchers for molecular docking study. AutoDock softwear is helpful for researcher as thissoftwear is predicting docking score which is corelate with *Invivo* or *In vitro* activity. Sp this is the ultimate tool in drug discovery.

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REVIEW ARTICLE

Organization of Swiss Dock: In study of Computational and Molecular Docking Study

Neha Subhash Patil*, Sachin H. Rohane
Department of B. Pharmacy at Yashoda Technical Campus, Satara.
*Corresponding Author E-mail: **nehap2761@gmail.com**

ABSTRACT:
In the present era, many fields of research are showing great importance. Apart from the applications researchers have grown their interest in pharmaceutical application. Protein play and important side in study of various in - vitro and in - vivo studies to understand the action of drugs. Docking programs have a wide range of applications ranging from protein Engineering to the drug design. Swiss dock software was guide to authors to predict the molecular interactions that may occur between a target protein and a small molecule. After review it was analysed that Swiss dock are organised for UV-VIS spectroscopy, Synthesis, crystal, structures, etc. This article presents Swiss Dock, a web server dedicated to the docking of small molecules on target proteins. It is based on the EADock DSS engine, combined with setup scripts for curating common problems and for preparing both the target protein and the ligand input files. An efficient Ajax/ HTML interface was designed and implemented so that scientists can easily submit dockings and retrieve the predicted complexes. For automated docking tasks, a programmatic SOAP interface has been set up and template programs can be downloaded in Perl, Python and PHP. The web site also provides an access to a database of manually curated complexes, based on the Ligand Protein Database.

KEYWORDS: Molecular Docking, Swiss Dock, Drug Discovery.

INTRODUCTION:
Molecular docking is prediction of the binding affinity To achieve an optimize Conformation for both receptor and ligand and relative orientation between protein and ligand such that the free energy of the overall system minimized Molecular docking is one of the most frequently used methods in structure based drug design, due to its ability to predict the binding conformation of Small molecule ligands to the appropriate target binding site. Molecular docking has become an increasingly important tool for drug discovery Programs based on different algorithm were developed to perform molecular dockings studies which have made docking an increasingly important tool in pharmaceutical research.

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The aim of molecular docking is to give a prediction of the ligand-receptor Complex structure using computation methods Swiss Dock a web Service to predict the molecular interaction that may occur between target protein and a small molecule many binding modes are generated either in a box or in a vicinity of all target cavities [1-3].

Computer aided Drug Design:
Drug design depends on knowledge of 3D structures of biomolecules that is known as structural aided drug design. in addition to small molecules biopharmaceutical induces peptides and especially therapeutic antibodies are increasingly important doss of drug and computational method for improving affinity selectivity and stability been developed.

Most docking programs are Complex computational machineries and require specific additional Sampling or Scaring parameters to which they might be very sensitive while convenient default values are often a proposed good understanding of the user manual and the original

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article is always required in order to achieve meaningful prediction.

Swissdock:

Swiss Dock is the docking web server that addresses limitations described above. Well a the structure of the target protein, as well as that of the ligand, can be automatically prepared for docking. Allcalculation performed on the server side, so that docking runs do not require any Computational power from the user.

A target protein structure can be determined either by specifying its identifier from the protein data Bank. Since the calculation are performed in the CHARMM force field, Swiss dock Supports the uploading of CHARMM. Formatted files in addition to the Commonly used PDB format protein Structure can be uploaded as a set of protein structure file, Coordinate file and extra topology and parameter files if needed. Once the target protein structure has been defined it is immediately prepaid for used with CHARMM, and the Curated structure can be downloaded and reviews prior to the docking assay if needed. The performance of the backend of Swissdock was assessed by a blinding docking assay on 251 test Complexes taken from the ligand protein Database with different presents available from the web interface. The performance of Swiss Dock depends on the number of free dihedrals of the ligand.

Influence of the flexibility of the success rate observed with SwissDock the docking engine of SwissDock redocking assay Carried out on 251 protein ligand Complex.

Table No. 01

Max No. of routable bond of the Ligands	FDA Approved Drug (in %)	SR0 (%)	SR5 (%)
5	63	84	93
10	93	77	86
15	99	69	83
20	100	66	81

The fraction of the surface of the ligand which becomes buried upon complexation also has Significant effect the higher this fraction, the easier it is for Swiss Dock to identify the binding pocket, and therefore to dock the ligand inside.

Table No. 02

Influence of the fraction of the Ligand which is buried upon complexation (% BS) on the same data set.

Min % BS	SR0 (%)	SR5(%)
95	82	95
90	70	85
85	66	80
80	62	75

Swiss Dock Input Files

- Since docking assays are carried out in the CHARMM 22 /27 all-hydrogen force field.
- Target proteins and ligands that have been uploaded as CHARMM- Formatted files Can be used.
- Protein and ligands that have been submitted in PDB or be mold to format respectively have to be Converted prior to the docking itself.

Computer Aided Drug Design:

Simply rational design is the inventive process of finding new medication based on biological target the drugs are commonly organic small moleculesthats activates or inhibits function of biomolecules such like a protein. That is further give the therapeutic action to the patient. Basic in that isdrug design means the invole in molecules that complementry in shape and size of biomolecules. They bind with each other and form the bond. Drug design not relies on computer modelling. this type of modelling callled as computet aided drug design. Drug design depends on knowledge of 3D structures of biomolecules that is known as structural aided drug design. in addition to small molecules biopharmaceutical indudes peptides ans especially therapeutic antibodies are incresingly important dass of drug and computational method for improving affinity selectivity and stability been developed.

Drug design also known as efforts to develop a new drug by molecular modification of lead compound for optimization of desired efferts and minimization of side effects.

- **Web interface Inputs:**

Only three steps are required to start a docking assay through the web interface of SwissDock: users must define a protein structure, one or several putative ligands and docking parameters. They are guided throughout this short and simple submission process by a comprehensive contextual help. As mentioned above, several sample files are supplied to users and can be directly uploaded into the form simply by clicking on a link. The corresponding sample output files are also provided.

- **Target selection:**

A target protein structure can be determined either by specifying its identifier from the Protein Data Bank (15) or by uploading structure files. The first option allows users who are not familiar with 3D structure files to start a docking assay with only a PDB code. If several PDB records are available for the same target, those with a high resolution and a ligand similar to the one that will be docked should be considered first.

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- **Ligand selection:**

A ligand can be selected either by specifying its identifier from the ZINC database (23) or by uploading structure files. The former possibility allows users who are not familiar with 3D structure files to start a docking assay with only a ZINC accession code (AC). The latter allows uploading several ligands at once or uploading ligands that are not present in the ZINC database. As for the target protein, SwissDock not only supports the widely used Mol2 format, but also the direct upload of CHARMM input files describing the ligand.

Review of Litreture:

Sabrin R. M. Ibrahim and Khalid Z. Al-shaliand et al used SwissDock ADME for Molecular Docking studies of the tested metabolites estimated to shade up rational explanation of α – amylase inhibitory activity result the pharmacokinetic parameter [4].

Mohammad Kabrineand et al used SwissDock in article to investigate the possible mechanism by which selected drugs act an silico theoretical molecular docking approach was used, during this study they stimulated the binding mode of N3 against 6lu7 crystal structure using SwissDock to ensure the effectiveness of Dock result and to compare result produced by several drugs to those of N3 [5].

Kerry A. Ramshottom et al used SwissDock programme to investigate that if the software cooled accurately dock the abacavir back into the crystal structure for the protein arising from the known risk allele and if the software is able to distinguish between the HLA-associated and known HLA associated allele [6].

Long Ding and et al used SwissDock software to discover Bioactive peptide silico method peptide would be experimental in-vitro to identify the activity [7].

Dae Hawn-Kim and et al used SwissDock stimulation analysis of unbiased blind docking it was determined the top score predicted blind side for SGI-1027 and M\M to localize the binding region on PrP. The result indicates CHI-1027 interacted with and regions on PrP [8].

Jesus Campagna and et al used SwissDock for evaluation of an Allosteric BACE inhibiter peptide to identify mimetic that can interact with the loop and region of the Enzyme and prevent APP cleavage and to elucidate the mechanism of peptide 65007 allosteric inhibition in silico experiment were performed first by conducting molecular docking in SwissDock with 65007 and comparing the model to crystal structure of the genetech Ab and BACE [9].

Layla K. Mahdi and et al used SwissDock experiment with GlpO model and its Ligands shows surface representation and the carbo-hydrate ligands as sticles. A predicted binding modes for LNT with a surface representation of GlpO model [10].

ElahelKashoni – Amin and et al used SwissDockfor the active site celef is quite extended, some poses were found to occur for the ligand in this location, However a second putative interacting site was found that is located in the entrance of the central beta-barrel of the enzyme. It should be seen that this location was detected by Swiss Dock [11].

Kankana Das and et al used SwissDock in compatible lipid lingad was then docked with proteins that are available as original PDB files were SwissDock interface the results were viewed and analysed [12].

Flavia S. Darquiand et al used SwissDock for putative KpFat A and KpFatB protein structure when moldedbu using homology modling using the Swiss model workspace. Based on their sequence the semi-colum zero-Acpthioesterace crystal structure from G. Californica as a term plate and default primary parametaFurthemore molecular docking was performed [13].

Christina E. Smith and et alused SwissDock for performing the docking of NSAIDs with Capase-3 was performed [14].

Jamal Quazzaniand et al used SwissDock in Silico studies for the docking of GP269, target was prepared from the X-ray protein structure of the crystalized complex. Polar hydrogen atom were added to the protein, The docking of GP269 into the structure of Tb6PGL was performed using the Swiss Dock [15].

CONCLUSION:

From Reviewing the above literatures, it was found that the Swiss Dock Software is used to predict the molecular interaction that may occur between a target protein and a small molecule.

The Swiss Dock web server aims at providing a wide scientific community with a free and user-friendly, yet stateof-the-art protein/small molecule, docking tool. The automatic setup of protein and ligand structures, the different parameter presents and the convenient visualization and analysis of docking predictions makes it accessible to a wide audience. The EADock DSS engine behind SwissDock is especially suited for drug design, with very good success rates for small and relatively rigid ligands with less than 10 flexible rotatable bonds: the most favourable predicted BM is

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found within 2 A˚ to the crystal structures for 77% of the 251 test complexes, and for 86% of them, such a correct BM is found within the five most favourable ones. This performance is even increased if the ligand can be buried in a well-defined binding site of its target protein.

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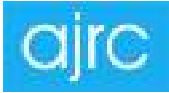

REVIEW ARTICLE

Drug Designing in Discovery Studio

Bhagyashree L. Jejurikar*, Sachin H. Rohane
Department of Pharmacy, Yashoda Technical Campus, Satara 415015 India.
*Corresponding Author E-mail: **bljejurikar@gmail.com**

ABSTRACT:
The process of drug development and drug discovery is very challenging expensive and time consuming. It has been accelerated due to devlopment of computational tools and methods. In current situation lots of molecular docking software are available in the market, during lireature survey it was found that Discovery Studio is suit of software for stimulating small molecule and macromolecule system. It is developed and distributed by Accelarys. It helps to Investigate and test hypothesis in silico prior it costly experimental implimentation thus reducing the time and expense involved in bringing products to market. it is developed and distributed by Dassault system BIOVIA (formerly Accelerys). During literature survey it was found that Discovery Studio software was efficiently guided to as author regarding mainly biological activities like anti-inflammatory activity, anti-tubercular activity, anti-bacterial activity, anti-viral activity, anti-diabetic activity and anti-oxidant activity.

KEYWORDS: Molecular docking, Drug discovery, Discovery studio.

INTRODUCTION:
Molecular docking is one of the most frequently used methods in structure based drug design, due to its ability to predict the binding- confirmation of the small molecule ligands to the appropriate target binding site. Discovery and development of a new drug is generally known as a very complex process which takes a lots of time and resources. So now a days computer aided drug design are used very widely to increase the efficiency of the discovery and development courses. CADD are evaluated as promising technique according to they need, in between all these structure – based drug design and ligand – based drug design. As very efficient and powerful technique in the drug Discovery and development. These both methods can be applied with molecular docking to the virtual screening for lead identification and optimization. Molecular docking is a key tool in structural molecular biology and computer assisted drug design.

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Discovery studiosoftware is suit for stimulating small molecule and Macromolecule system. The product suite as astrong academic collaboration programme, supporting scientific research and makes use of a number of software algorithms developed originally in the scientific community, including CHARMM, MODELLER, DELPHI, ZDOCK, DMol3 and more [1-3].

REVIEW OF LITERATURE:
Qinggang Meng and et al used Discovery Studio Software check the Rizoma Atractylodis and Rhizoma Atractylodis Macroephalae herbal pairs against type 2 diabetes mellitus. the interaction between targets and ligands were observed and analazyed, according to CDocker interaction energy, most compounds from the herbal pair had good binding activities with receptor and nine compounds had even higher scores than those of the original ligands [4].

Shola Elijah Adeniji and et al used Discovery Studio stimulated Software to check the Anti tubercular modeling, molecular docking stimulation and insight toward computational design of novel compounds as potent antagonist against DNA gyrase receptor. Tuberculosis continue to be critical health problem causing death and illness among millions of people yearly and ranked the second leading cause of mortality

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among the communicable infections in the world. This work accessed the application of modelling technique to predict the inhibitory activity of some prominent compounds which been reported to efficient against mycobacterial tuberculosis [5].

Sabir Ali and et al used Discovery Studio4.0 accelrys Software to check anti bacteriak, anticancer, and molecular docking studies of macrocyclic metal complexes of dihydrazide and diketone. this studies suggest an octahedral geometry for all complexes, compounds found active against B, Substillis and S,aureusand P, aeruginosa and E.coil bacteria, Zn (II) complex showed significant anticancer activity against squamous cell carcinoma cell tested by MTT assay method. Molecular docking studies with EGFR tyrosine kinase were also carried out . all these results show that some of synthesized compound have remarkable antibacterial and anticancer property [6].

Mehmet Gokhan Caglayan and et al used Discovery Studio Software to check the Electrochemical ,Spectroscopic and molecular docking studies of the insteraction between the anti –retroviral Indinavir andds DNA in this study electrochemical and DNA biosensor was developed using a straightforward methodology to investigate the ineraction of indinavir with calf thymus double –stranded deoxyribonucleic acid for the first time. The obtained results can offers insights into the inhibitory activity of indinavir, which could heip to broaden its application, thus indinavir can be used to inhibit other mechanisum and /or hallmarks of viral diseases [7].

Dominic Agyei and et al used discovery studio software 2019 to study physicochemical characterization and drud likeness evaluation of hypotensive peptides encrypted in flaxseed proteome.in this study, hypotensive peptides derived from mature flaxseed protein sequences were predicted in silico using BIOPEP-UWM with nine protease, three each form digestive, plant and microbial sources. In silico prediction of adsorption, digestion, metabolism, excetion and toxicity (ADME/Tox) profile based on physiochemical properties and Lipinski's rule of five showed that the peptides were non toxic and had desirsble drug like properties [8].

Shola Eljiah Adenjiand et al used Discovery Studio stimulated Software to check Quantum modeling molecular and evaluation of some selected quiniline derivative as antitubercular agents: discovery studio visualize software. Ligand - receptor interaction between quinoilne derivatives and the receptor (DNA gyrase). Docking study indicates that compounds 10 of the derivative with promising biological activity have the ulmost binging anti- tubercular drugs with more efficient activities [9].

Nasser Abdulatif Al- Shabib and et al used Discovery studio 4.0 software to investigate the effect of food additive dye "tartrazine" on BLG fibrillation under invitro condition. Molecular docking results ascertained that Tz binds at the hydrophobic cavityand interact with the key amino acid residues involved in the interaction with different ligands, the spectroscopic,microscopic and computational results electrostatic as well as hydrophobic interaction played a very important role in Tz–induced BLG fibrillation under invitro condition [10].

Jian Wang and et al used Discovery Studio Software to study Graphene/Feso4 namocomposite for effective removal of ten triazole fungicides from water solution . Tebucanazole as an example for investigation of the adsorption mechanism by experimental and molecular doking study. The study on adsorption kinetics and thermodynamics were done by taking tebuconazole as an example. Grapheme/Fe_3SO_4 was prepared and utilized as a adsorbent for removal of ten commonly used triazole fungicides in agriculture [11].

Deepu Mathew and et al used Discovery Studio V4.0 Software to check therapeutic molecule for multiple human diseases identified from pigeon pea (Cajanus Cajan L. Millsp) through GC- MS and molecular docking molecular mechanism behind the therapeutic potential of pigeon pea over the human disease such as rheumatoid arthritis, breast cancer, type II diabetes , malaria, measlesand sickle cell disease were revealed through GC-MS identified phyto – compound ligands with candidate protein [12].

Asif Husain and et al used Discovery Studio Software (version4.0,Accelryssoftware) check the molecular docking with COXI and II enzyme, ADMET screening and in vivo anti– inflammatory activity of oxadiazole, thiadiazole and triazole analogus of felbinae. Based on the core structure of felbinac drug, three series (4a-d,5a-d, 6a-n) of five membered heterocylic derivatives containing three heteroatoms were designed and synthesized starting from felbinac. The prepared molecules were the investigated for their anti-inflammatory, ulcerogenicity, and analgesic in experimental animal [13].

Maryam A. Jordaan and et al using Discovery Studio visualizer Software to check virtual screening and DFT calculation of FDA approved compounds similar to the non – nucleoside reverse transciptase inhibitor (NNRTI) efavirenz the compounds were subjected to screening by analyzing their drug likness, such as Lipinski's rule of

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five and ADME properties. Study showed that lovastatin and simvastatin might be considered as lead compounds for further development for COVID -19 main perotease inhibitor [14].

Assia Belhassan and et al used Discovery Studio2016 Software to study novel triazolo- benzodiazepine analogues as antidepressant targeting by molecular docking and ADMET properties prediction. Docking studies suggested that eleven ligands interacted within activesiteof Drosophila melangaster dopamine transporter (Ddat)) (PDBED:4M48) [most ligands formeH-bond with amino acid phe43, Asp46, Asp475, Tyr123, Ser421 and Gln also exhibited Pi and Pi-Pi. In silico ADME evaluation of compounds shoed more than 96% intestinak absortion for all compound [15].

Mohommad k.Parvez and et al used discovery studio software to check plant derived antiviral drugs as novel hepatitis B virus inhibitors: Cell culture and molecular docking study. Docking of lamivudine indicatedstrong interaction with the modeled HBV pol active site residues that formed stable complex, similarly all the docked antiviral compounds formed very stable complexes with anti-HBV pol. Taken together,our data suggest the anti-HBV potential of thetested natural compounds as novel viral pol RT inhibitors [16].

Hanine Hadni and et al used to discovery studio software to check molecular docking andthe antimalarial activity of hybrids 4-anilino-qinoline-triazines derivatives with thewild-type and mutant receptors ofof-DHFR docking studies were performed for previously reported 4-aniloquinoline and 1,3,5-triazines based molecular hybrids. The docking result revealed that these molecular specifically with SER108 and 1LE164 in the pf-DHER binding pocket as that of best active compounds but also showed additional interactions with LEU40 and GLY44 [17].

Aliyu Wappah Mahmud and et al used discovery studio software check QSAR and molecular docking studies of 1,3,dioxoisoindoline-4-aminoquinoline as potent antiplasmodium hybrid compounds. The docking result indicates strong binding between 1,3-dioxosoindoline-4-aminoquinoline and plasmodium falciparum lactate dehydrogenase (PFLDH) ,and revealed the important of the morpholinyl substituent and amide linkerin inhibiting PFLDH. These results could serve as a model for designing novel 1,3-dioxoisoindoline-4-aminoquinolines as inhibitors of PFLDH with higher antiplasmodial activities [18].

K.Jayasheela and et al used discovery studio to check the conformational and spectroscopic characterization, charge analysis and molecular docking profiles of chromone-3-caboxylic acid using a quantum hybrid computational method. The spectroscopic profile of chromone-3-carboxylic acid (abbreviated as 3CA) was exmined using FT-IR, FT-roman,UV,1H and 13C NMR technique. Result of the docking study identified the sugar phosphate inhibitor activity of the target molecular (C3CA) [19].

Mariana Spetea and et al used discovery studio (version3.0) software to check structure activity relationship. Explorations of 14-oxygenated N-Methylmorphonan-6-ones as potent μ-Dpioid receptors against. The crucial role of relative orientation of the ligand in the u or binding site, influencing the property of critical non-covalent interaction that are required to facilitate ligand/-μ or activation by the 14-oxygenated N-methylmorphinan-6-ones,which should be useful for guiding drug design.

Shola Elijiah Adeniji and et al using Discovery Studio Visualizer software. To check the theoretical modeling and molecular docking stimulation for investigating and evaluatiny some active compounds as potent anti tubercular agents against MTB CYP 121 receptor. Docking studies revealed the best molecule with docking scores of–13.7 kcal/mol which formed H- bond and hydrophobic interaction with amino acid residue M. Tuberculosis cytochromes (MTB CYP 121) [20].

Shola Elijiah Adeniji and et al used Discovery Studio Visualizer software check the In silico study for evaluating the binding mode and interaction of 1,2,4-triazole and it's derivatives as potent inhibitors against Lipoate protein B (Lip B) research has shown that the binding affinity of these compounds wery found to be better than the recommended anti–mycobacterium drugs; isoniazid (-14.6kcal/mol) and ethambutol (- 5.8kcal/ mol). This study provide valuable approach for designing and synthesizing more potent anti–mycobacterium tuerculosis derivatives [21].

Ana- Maria Udrea and et al used Discovery Studio Visualizer software to check Laser irradiated phenothiazines: New potential treatment for COVID - 19 explored by molecular docking . In this study predict , using molecular docking, the binding affinity to 15 phenothiazines (antihistaminic and antipsychotic drugs) when interacting with the main protease SARS - coV-2 . Results reveal that thioridazine –and its identified photo products (mesoridazine and sulforidazine) have high biological activity on the virus main protease [22].

CONCLUSION:

This review totally focused on the drug designing in Discovery Studio software and it's different versions such as Discovery Studio V 4.0, Discovery Studio 2016,

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Discovery Studio 4.0 Accelrys. This software also useful to check the various biological activities and therapeutic activities such as Anti -inflammatory, Anti- tubercular, Anti-diabetic and Anti- oxidant activity etc.

After literature review it was seen that number of researchers suggest Discovery studio software for protein – ligand interaction and the interaction between target and ligands were observed and analyzed.

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RESEARCH ARTICLE

Role of Aminated derivatives of Natural Gum in Release Modulating Matrix Systems of Losartan Potassium: Optimization of Formulation using Box-Behnken Design

Shankar B. Kalbhare[1]*, Dr. Vivek Kumar Redasani[2], Mandar J. Bhandwalkar[3], Rohit K. Pawar[3], Avinash M. Bhagwat[1]
[1]Assistant Professor, YSPM's Yashoda Technical Campus, Satara (M.S.) India.
[2]Director, YSPM's Yashoda Technical Campus, Satara (M.S.) India.
[3]Assistant Professor, Late Narayandas Bhawandas Chhabada Institute of Pharmacy, Satara (M.S.) India.
*Corresponding Author E-mail: **kirankal786@gmail.com**

ABSTRACT:
The aim of the present research work was to systemically device a model of factors that would yield an optimized release modulating dosage form of an anti-hypertensive agent, losartan potassium, using response surface methodology by employing a 3-factor, 3-level Box-Behnken statistical design. Independent variables studied were the amount of the release retardant polymers – aminated fenugreek gum (X_1), aminated tamarind gum (X_2) and aminated xanthan gum (X_3). The dependent variables were the burst release in 15 min (Y_1), cumulative percentage release of drug after 60 min (Y_2) and hardness (Y_3) of the tablets with constraints on the Y_2 = 31–35%. Statistical validity of the polynomials was established. In vitro release and swelling studies were carried out for the optimized formulation and the data were fitted to kinetic equations. The polynomial mathematical relationship obtained $Y_2=32.91-2.29X_1-5.68X_2-0.97X_3+0.20X_1X_3-0.005X_2X_3-0.92X_1^2-1.89X_2^2$ explained the main and quadratic effects, and the interactions of factors influencing the drug release from matrix tablets. The adjusted (0.9842) and predicted values (0.9600) of r^2 for Y_2 were in close agreement. Validation of the optimization study indicated high degree of prognostic ability of response surface methodology. The Box-Behnken experimental design facilitated the formulation and optimization of release modulating matrix systems of losartan potassium.

KEYWORDS: Release modulating matrix tablets; Amination of natural polymers; Losartan potassium; Box Behnken statistical design; Response surface methodology.

INTRODUCTION:
Oral drug delivery is the most preferred and appropriate preference as the oral route provides maximum active surface area amongst all drug delivery system for administration of a various drugs. Significance of these dosage forms is due to awareness to toxicity and ineffectiveness of drugs when administered by oral conventional method in the form of tablets and capsules. Developing oral sustained release matrix tablets for drug with constant release rate has always been a challenge to the pharmaceutical technologist [1]. Usually conventional dosage form produces wide range of

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variation in drug concentration in the bloodstream and tissues with consequential undesirable toxicity and poor efficiency, poor bioavailability. So the maintenance of concentration of drug in plasma within therapeutic index is very significant for effective treatment and high bioavailability [2],[3]. Drug release through various matrix system is determined by Water penetration, Polymer swelling, Drug dissolution, Drug diffusion, Matrix erosion have been utilized as formulation sustained release drug delivery. Sustained release dosage forms offer better control of plasma level less dosage frequency, less side effect, increased efficacy and constant delivery [4].

A polymer is a large molecule (macromolecules) composed of repeating structural units. These subunits are typically connected by covalent chemical bonds. Both synthetic and natural polymers are available but the use of natural polymers tier pharmaceutical applications is attractive because they are economical, readily available and non-toxic. They are capable of chemical modifications, potentially biodegradable and with few exceptions, also biocompatible [5]. Derivatization of native polymer led to enhancement in bioadhesive and drug release characteristics [6]. Recently, chemical modification or derivatization of natural polysaccharides has been reported to improve the functional properties of native gums. Reports in the literature suggest that the derivatives of polysaccharides (amine, thiol, carboxymethyl) can be employed to manipulate swelling, bioadhesion and drug release [7]. A few examples of polysaccharide derivatives already reported in literature include aminated fenugreek gum [6], aminated tamarind kernel polysaccharide [8] and aminated xanthan gum [9]. Polymeric material have fulfilled different roles such as binders, matrix formers or drug release modifiers, film coating formers, thickeners, viscosity enhancers, stabilizers, disintegrants, Solubilisers, emulsifiers, suspending agents, gelling agents and bioadhesive.

Losartan potassium (LP) is a potent, highly specific angiotensin II type 1 (AT1) receptor antagonist with antihypertensive activity [10], [11]. It is readily absorbed from the gastrointestinal tract with oral bioavailability of about 33% and a plasma elimination half-life ranging from 1.5 to 2.5 h. Administration of LP in a controlled release dosage form with dual release characteristics i.e., burst release followed by an extended release over 8 h, would be more desirable as these characteristics would allow a rapid onset followed by protracted anti-hypertensive effects by maintaining the plasma concentrations of the drug well above the therapeutic concentration [12].

Response surface methodology (RSM) is one of the popular methods in the development and optimization of drug delivery systems [13]. Based on the principles of design of experiments (DOE), the methodology involves the use of various types of experimental designs, generation of polynomial mathematical relationships and mapping of the response over the experimental domain to select the optimum formulation [14]. Central composite design (CCD), 3-level factorial design, Box Behnken design and D-optimal design are the different types of RSM designs available for statistical optimization of the formulations [13]. Box-Behnken statistical design is one type of RSM design that is an independent, rotatable or nearly rotatable, quadratic design having the treatment combinations at the midpoints of the edges of the process space and at the center. Additionally, it requires fewer experimental runs and less time a and optimized thus provides a far more effective and cost-effective technique than the conventional processes of formulating and optimization of dosage forms [15].

The current study aimed at developing and optimizing an oral release modulating matrix tablet of LP using computer aided optimization technique i.e. Box Behnken statistical design with constraints on cumulative percentage release of drug after 60 min (31–35%). The Independent variables for the present study were: amount of release retardant polymers – aminated fenugreek gum *(X_1)*, aminated tamarind gum *(X_2)* and aminated xanthan gum *(X_3)*. The dependent variables studied were the burst release in 15 min *(Y_1)*, cumulative percentage release of drug after 60 min *(Y_2)* and hardness of the tablets *(Y_3)*.

MATERIAL AND METHODS:

Materials:

Losartan potassium was provided as a gift sample by Viraj Pharmaceutical. (Mumbai, India). Carbopol, magnesium stearate and microcrystalline cellulose were supplied by Thermosil Fine Chem industry. (Charhol). Feenugreek gum, tamarind gum and xanthan gum were purchased from Phyto Lifesciences Pvt Ltd. (Gandhinagar, Gujarat). Starch, isopropyl alcohol and ethylene diamine as a supplied by SD. Fine Chemicals limited. (Mumbai, India). Sodium borohydride was purchased from Karan enterprise. (Mumbai, India). All other reagent and solvents used were of analytical grade and used as received.

Amination of natural polymers:

In 3000ml water add 60gm of natural gum. To this solution add aminating agent ethylene diamine (25ml) with continuous stirring at constant temperature (20-60°C) for 6 hr). Then slowly add reducing agent sodium borohydride ($NaBH_4$) for 2 hr until formation of thick gel. Wash this gel several times with ethyl alcohol and

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collect the precipitate of aminated derivative [6], [8], [9]. Synthesized aminated polymer was studied under further parameter for determination of flow properties, chemical stability and thermal properties.

Preparation of compressed matrix:

Drug, carbopol (binder) and the MCC (diluent) were sifted through #40 manually and mixed well to ensure the uniformity of premix blend [16]. Several drug-diluents premixes were then mixed with the selected combination and ratio of hydrophilic polymers (Aminated FG, Aminated TG and Aminated XG), previously sifted through #40, for 5 min. Premix blend was wet granulated with isopropyl alcohol and the granules were sized through #18 and were dried at 45°C for 15 min. Dried LP granules were lubricated with starch and magnesium sterate. The tablets were compressed at an average compression weight of 250mg by cold compression technique on dialed hydraulic press (KBR press) at 12.0mm, circular, flat punches at compressional pressure of 5 tons with 15 s dwell time [17],[18].

Different formulations of Losartan potassium 100mg release modulating matrix tablets were prepared using the following excipients: AFG (7.5-22.5mg), ATG (10–30mg), AXG (12.5–37.5mg), carbopol (30mg), starch (10.25mg), magnesium sterate (1.75mg) and MCC (q.s. to 250mg).

Experimental design:

Box-Behnken statistical screening design was used to optimize and evaluate main effects, interaction effects and quadratic effects of the formulation ingredients on the in vitro release of LP sustained release formulations [17],[19]. A 3-factor, 3-level design used is suitable for exploring quadratic response surfaces and constructing second order polynomial models with Design Expert® (Version 12.0.1.0, Stat-Ease Inc., Minneapolis, MN). This cubic design is characterized by set of points lying at the midpoint of each edge of a multidimensional cube and center point replicates ($n = 3$). The nonlinear computer-generated quadratic model is given as,

$$\mathbf{Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^{\ 2} + b_{22}X_2^2 + b_{33}X_3^2}$$

Where Y is the measured response associated with each factor level combination; b_0 is an intercept; b_1 to b_{33} are regression coefficients computed from the observed experimental values of Y; and X_1, X_2 andX_3 are the coded levels of independent variables. The terms $X_1\ X_2$ and $X^{\ 2}$ $(i = 1, 2\ or\ 3)$ represent the interaction and quadratic terms, respectively. The selected dependent and independent variables are shown (Table 1, left column) along with their low, medium and high levels, which were selected based on the results from preliminary experimentation [20]. The amounts of Aminated FG (X_1), Aminated TG (X_2) and Aminated XG (X_3) used to prepare each of the 15 formulations are given (Table 2).

Table 1: Variables in Box Behnken design

Factor	Level used, actual (coded)		
	Low (-1)	Medium (0)	High (+1)
X1=AFG (%)	3	6	9
X2=ATG (%)	4	8	12
X3= AXG (%)	5	10	15
Dependent variables	Constraints		
Y1 = % Burst release in 15 min	$10 \le Y1 \le 15$		
Y2 = % Dissolution after 60 min	$31 \le Y2 \le 35$		
Y3 = Hardness (kg/cm2)	Maximize (range 3.5–5.5)		
*All percentages were calculated with respect to total tablet weight of 250mg			

Tablet assay and physical evaluation:

The tablets were assayed for drug content using methanol as the extracting solvent, and the sample were analyzed spectrophotometrically (Shimadzu- UV-1800, Japan) at 215 nm [17], [20]. Tablets were also evaluated for the hardness ($n = 6$) (Monsanto hardness tester), friability ($n = 6$) (Roche Friabilator, 100 rpm), weight variation ($n = 20$) and thickness ($n = 10$) (Vernier caliper).

Table 2: Observed responses in Box Behnken design for losartan potassium release modulating matrix tablet

Batch	Dependent Variables			Independent variables		
	X1 (%)	X2 (%)	X3 (%)	Y1 (%)	Y2 (%)	Y3 (kg/cm^3)
1	3	4	10	11.87	37.53	3.5
2	9	4	10	9.63	34.27	4
3	3	12	10	7.16	26.76	5
4	9	12	10	6.81	21.84	5.5
5	3	8	5	10.45	35.63	4.5
6	9	8	5	8.56	30.12	4
7	3	8	15	9.27	33.41	3.5
8	9	8	15	8.13	28.73	5.5
9	6	4	5	12.41	37.61	3.5
10	6	12	5	7.23	26.48	5.5
11	6	4	15	10.71	35.54	3.5
12	6	12	15	6.69	24.39	5.5
13	6	8	10	8.87	33.74	5
14	6	8	10	8.13	32.21	4
15	6	8	10	7.33	32.79	4.5

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In vitro drug release studies:
Dissolution studies were performed using the USP II, paddle-rotating method (Electrolab dissolution tester, Electro lab, India) at 37°C ± 0.5°C and 75rpm using 0.1 N HCl (2 hr) and phosphate buffered solution, pH 6.8 (PBS) (10 hr), as the dissolution media. Dissolution studies were carried out in triplicate, maintaining the sink conditions for all the formulations. A 5ml aliquot of sample was withdrawn at regular time intervals, filtered and assayed spectrophotometrically at 205.3nm. The cumulative % drug release was calculated for the formulations [21],[22].

Swelling and erosion studies:
Swelling and erosion studies of the matrix tablets were carried out under conditions identical to those described for the dissolution testing. After 2 hr in 0.1 N HCl and 6 hr in phosphate buffer, pH 6.8, the tablets were removed, gently wiped with a tissue paper to remove surface water and Scanning Electron Microscopy (SEM) study of the hydrated swollen tablets was carried out [17],[23]. Water uptake and mass loss were determined gravimetrically according to the following equations;

Degree of swelling (water uptake)=
(Wet weight - Original dry weight)/(Original dry weight)

Erosion (% mass loss)=
(Original weight - Remaining dry weight)/(Original weight)

Thermal properties:
Differential scanning calorimetry (DSC) experiments were performed on drug, excipients and the optimized formulation using DSC (Perkin–Elmer, Norwalk, CT). The instrument was calibrated using indium standards. Accurately weighed samples (5–10mg) were hermetically sealed in flat bottom aluminum pans and heated from 48 to 300°C at a rate of 10°C per min under an atmosphere of nitrogen. Thermograms were normalized and rescaled as needed before overlapping [11],[24].

Fourier transforms infrared spectroscopy (FTIR):
FTIR studies were performed on drug, excipients and the optimized formulation using Shimadzu FTIR (Shimadzu Corp., India). Background spectrum was collected before running each sample. The samples were analyzed between wavenumbers 4000 and 400 cm^{-1}.

Optimization data analysis and validation of optimization model:
Statistical validation of the polynomial equation generated by Design Expert® was established on the basis of ANOVA provision in the software. A total of 15 runs with triplicate center points were generated. The models were evaluated in terms of statistically significant coefficients, standardized main effects (SME) and R^2 values. Various feasibility and grid searches were conducted to find the compositions of optimized formulation. Various 3-D response surface graphs were provided by the Design Expert software. By intensive grid search performed over the whole experimental region, nine optimum checkpoints formulations were selected to validate the chosen experimental domain and polynomial equations. The optimized checkpoint formulations were prepared and evaluated for various response properties. The resultant experimental values of the responses were quantitatively compared with that of the predicted values. Also, linear regression plots between actual and predicted values of the responses were produced using MS-Excel.

RESULTS AND DISCUSSION:

Characterizations of derivetized natural polymers:
The synthesis polymers are characterized using ATR-FTIR, DSC and XRD studies. In this study, synthesis polymers are confirmed by ATR-FTIR study. The ATR-FTIR study of aminated fenugreek gum, aminated tamarind gum and aminated xanthan gum is confirmed by the appearance of a new peak at 3271 cm^{-1}, 1639.49 cm^{-1} and 2899.01 cm^{-1} respectively corresponding to NH_2 group.

Aminated fenugreek gum:
The DSC thermograms of AFG, in that the AFG shows the broad endothermic peak at 9.94°C with heat of fusion 38.33 J/g, and the exothermic peak does not appeared. These transitions occur at a lower temperature as compared with fenugreek gum. The endothermic peak is due to the loss of water content in polymer (Bassi & Kaur, 2015). The disappearance of exothermic peak due to complete degradation of polymer backbone is observed. These peak shows low thermal stability as compared to fenugreek gum i.e. decreased availability of OH groups for intra-molecular hydrogen bonding. The Tg of AFG was also observed high in AFG (47.34°C) as compared to FG (47.95°C), indicating a high degree of Crystallinity of polymer.

DSC of Aminated tamarind gum:
The DSC thermograms of aminated tamarind, in that the aminated tamarind shows endothermic peak at 82.40°C and 394.50°C with heat of flow 1.002 w/g and 9.231 w/g or the exothermic peak does not appeared. The endothermic peaks are due to the loss of water content in polymer. The disappearance of exothermic peak was 365.35°C and heat of flow 9.639 w/g, due to complete degradation of polymer backbone. The melting point was showed by 276.49°C.

DSC of aminated xanthan gum:
The DSC thermograms of aminated xanthan gum, in that the aminated xanthan gum shows endothermic peak at 71.74°C and 541.66°C with heat of flow 1.644 w/g and 9.424 w/g or the exothermic peak does not appeared.

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Table 3: Flow properties of natural gum and derivetized gum

Polymer	Bulk density	Tapped density	Angle of repose	Compressibility index (%)	Hausner's ratio
Fenugreek Gum	0.37	0.41	33.6	18.6	1.22
Aminated Fenugreek Gum	0.39	0.45	35.9	13.33	1.15
Tamarind Gum	0.37	0.41	15.4	9.75	1.1
Aminated Tamarind Gum	0.41	0.55	18.2	25.45	1.34
Xanthan Gum	0.38	0.45	16.7	15.55	1.18
Aminated Xanthan Gum	0.43	0.52	19.1	17.3	1.2

*All value calculated in average of five reading.

The endothermic peaks are due to the loss of water content in polymer. The disappearance of exothermic peak was 430.05°C and heat of flow 11.82 w/g, due to complete degradation of polymer backbone. The melting point was showed by 277.20°C.

The X-ray ray differactograms (XRD):
The X-ray differactograms of aminated fenugreek gum, aminated tamarind gum and aminated xanthan gum. The diffractions curve of aminated fenugreek gum, aminated tamarind gum and aminated xanthan gum was typical of amorphous material with no sharp peaks.

Flow properties and physicochemical evaluation of aminated polymers:
The bulk density, tapped density and angle of repose of synthesized polymers were increased as compared to natural polymer due to reason of chemical modification was done in natural polymer. Compressibility index and Hausner's ratio describe the flow properties of natural polymers and derivetized polymers. Observations as per compressibility index the aminated fenugreek gum shows good flow properties as compare to natural fenugreek gum and aminated tamarind gum shows poor flow properties as compare to natural tamarind gum. In case of aminated xanthan gum compressibility index is partially an increases shows good flow property. The values of Hausner's ratio are < 1.25, shows good flow. Here all derivetized polymers show good flow properties except aminated tamarind gum. Evaluation parameters like bulk density, tapped density, angle of repose, compressibility and Hausner's ratio was carried out for the natural polymers and derivetized polymers and was found to be within the limit as given in Table 3.

Drug content and physical evaluation:
Drug content of the formulations was assayed spectrophotometrically at 215nm. Assayed content of drug in various formulations varied between 98.23% and 100.30% (average 99.35%). Tablet weights varied between 249.29mg and 250.30mg (average 249.93 mg), hardness between 3.5 and 5.5kg/cm^2 (average 4.46 kg/cm^2), thickness between 3.09 and 3.12mm and friability ranged from 0.49% and 0.87% (average 0.73%). Thus all the physical parameters of the compressed matrices were found to be practically within controls.

Fitting of data to model:
A three-factor, three-level Box-Behnken statistical experimental design as the RSM requires 15 experiments (Polynomial analysis). The independent variable and the response for all 15 experimental run are given in Table 2. Eleven batches showed the burst release *(Y1)* of less than 10% and the range of *Y1* for all batches was 6.69–12.41%. The ranges of other responses, Y2 (% dissolution after 60 min) and *Y3* (hardness of the tablets, kg/cm^2), were 21.84–37.53% and 3.5–5.5kg/cm^2, respectively. All the responses observed for 15 formulations and analyzed with polynomial equation of statistics analysis [17] were simultaneously fitted to first order, second order and quadratic models using Design Expert® and the comparative values of R^2, S.D. and % C.V. are given in Table 4 along with the regression equation generated for each response. Responses *Y1*, *Y2* and *Y3* were found to follow linear, quadratic and second order model respectively (Table 4, right column). Only statistically significant ($p < 0.05$) coefficients are included in the equations. A positive value represents an effect that favors the optimization, while a negative value indicates an inverse relationship between the factor and the response. It is evident that the Aminated fenugreek gum *(X1)*, Aminated tamarind gum *(X2)* and Aminated xanthan gum *(X3)* have negative effects on the responses *Y1* and *Y2* in the following order;

ATG (X2) > AFG (X1) > AXG (X3)

Coefficients with higher order terms or more than one factor term in the regression equation represent quadratic relationships or interaction terms, respectively. It also shows that the relationship between responses and factors is not always linear. Used at different levels in a formulation or when more than one factors are changed simultaneously, a factor can produce different degree of response. The interaction effect of *X1* was seen with *X2* and *X3* for response *Y2*; and between *X1* and *X3* for response *Y3*. *X2* also showed a higher quadratic effect as compared to *X1* on response *Y2*. Percentage burst release *(Y1)* and hardness of the tablets *(Y3)* were found to fit the linear and second order models, respectively. In absence of the quadratic effects, *Y1* was mainly dependent upon the amount of ATG. For *Y3*, the critical parameters were found to be the AFG and the ATG.

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Table 4: Summary of regression analysis of *Y1*, *Y2* and *Y3*

Model	R^2	Adjusted R^2	Predicted R^2	S. D.	% C. V.	Remark
Response (Y1)						
Linear model	0.8805	0.848	0.8081	0.7093	7.98	**Suggested**
Second order	0.9101	0.8427	0.7901	0.7214	8.12	-
Quadratic model	0.9653	0.9028	0.7965	0.5672	6.39	-
Response (Y2)						
Linear model	0.9441	0.9288	0.9046	1.29	4.1	-
Second order	0.9467	0.9067	0.8189	1.48	4.7	-
Quadratic model	0.9944	0.9842	0.96	0.6069	1.93	**Suggested**
Response (Y3)						
Linear model	0.7514	0.6835	0.5217	0.4569	10.23	-
Second order	0.9206	0.861	0.8048	0.3028	6.78	**Suggested**
Quadratic model	0.9255	0.7915	0.5532	0.3708	8.3	-
Regression equation of the fitted model [a]						
Y1=8.88-0.70X1-2.09X2-0.48X3						
$Y2=32.91-2.29X1-5.68X2-0.97X3+0.20X1X3-0.005X2X3-0.92X1^2-1.89X2^2$						
Y3=4.5+0.31X1+0.87X2+0.06X3+0.62X1X3						
[a] Only the terms with statistical significance are included.						

Table 5: Standardized main effects of the factors on the responses

Factor	Standardized main effect of the factors on the responses		
	Burst release (Y1) linear model	**Dissol. 60 min. (Y2) quadratic model**	**Hardness (Y3) second order model**
X1	2.801441	10.7008	2.919371
X2	8.339521	26.4928	8.174239
X3	1.919137	4.52615	0.583874
*X1*X2*	-	-	-
*X1*X3*	-	0.683756	4.128614
*X2*X3*	-	0.01648	-
*X1*X1*	-	2.92189	-
*X2*X2*	-	5.98494	-
*X3*X3*	-	-	-
R^2	88.05%	99.44%	92.06
p- value of lack of fit	0.6624	0.791	0.9678

[a] Only term with statistical significance are included

Standardized main effects and reliability of the models:

Standardized Main Effects (SME), presented in Table 5, SME were calculated by dividing the main effects with the standard error of the main effects [17],[25]. Only statistically significant ($p < 0.05$) values are given. The larger SME value of *X2* suggested the paramount importance of ATG on drug release. R^2-value signifies the percentage of variability in responses that are fitted to the models. In the present study, the high R^2-value of > 99% represents the reliability of the design. Additionally, the *p*-values of lack of fit were greater than 0.05, which further strengthened the reliability of the models (Table 5).

Contour plots and response surface analysis:

Two-dimensional contour plots and three-dimensional response surface plots are presented in Figure 1 which is very useful to study the interaction effects of the factors on the responses. These types of plots show the effects of two factors on the response at a time. In all the presented figures, the third factor was kept at a zero level. Figure 1 (B) and (C) exhibits a nearly linear relationship of factor *X3* with factors *X1* and *X2*, in the form of almost straight line. Response surface plots show the relationship between these factors even more clearly. However, factor *X1* and *X2* have non linear relationship Figure 1 (A) and Figure 1 (D) shows that 39.5 % drug is released after 60 min *(Y2)* when both the AFG and ATG are at lowest level and the decrease in % drug release was polymer concentration dependent. Also the ATG resulted in greater reduction in % release at 12 % level as compared to the AFG at 9% concentration. This indicates a slight non-linear trend between the factors *X1* and *X2*. Figure 1 (E) and (F) show an increasing trend for *Y2* upon the replacement of either of AFG or ATG with AXG.

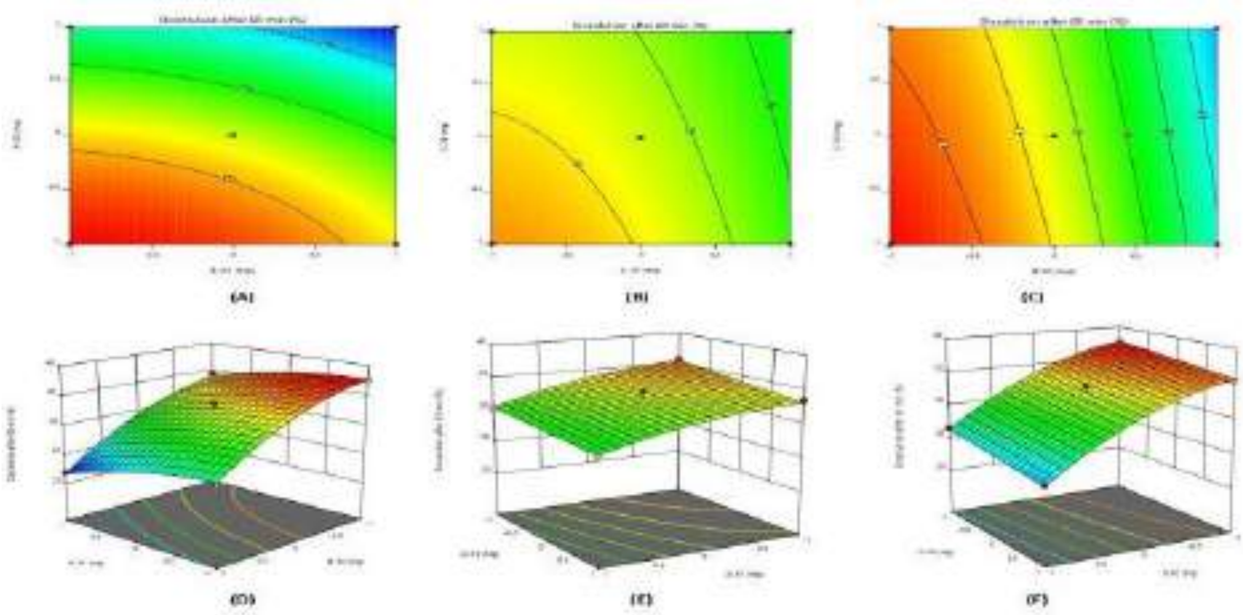

Figure1: Contour plot showing the effect of- (A) AFG (X1) and ATG (X2) on response Y2, (B) AFG (X1) and AXG (X3) on response Y2, (C) ATG (X2) and AXC (X3) on response Y2;
Response surface plot showing the effect of - (D) AFG (X1) and ATG (X2) on response Y2, (E) AFG (X1) and AXG (X3) on response Y2, (F) ATG (X2) and AXC (X3) on response Y2

Optimization:

The optimum formulation was selected based on the criteria of attaining the maximum hardness for tablets and applying constraints on Y1 ($10 \leq Y1 \leq 15$) and Y2 ($31 \leq Y2 \leq 35$). Upon 'trading off' various response variables and comprehensive evaluation of feasibility search and exhaustive grid search, the formulation composition with polymer levels of AFG- 37.40 mg, ATG- 10 mg and AXG- 24.91 mg, was found to fulfill the maximum requisite of an optimum formulation because of better regulation of % burst release and % dissolution after 1 hr time interval. The optimized formulation was found to release about 99.12% drug in sustained release manner for 12 hr. Study of the in vitro release profiles in 0.1 N HCl (for 2 hr) and in phosphate buffer, pH 6.8 (for 10 hr), of the formulations showed a burst release of 37.61% during 1 hr followed by a gradual release phase for about 10 hr. Figure 2 shows the complete dissolution profile of the optimized formulation. The optimization of the formulation was carried out from overlay plot. Overlay plot gives the area of interest or area of the experiment. In Figure 3 yellow region reflects the area of experiment.

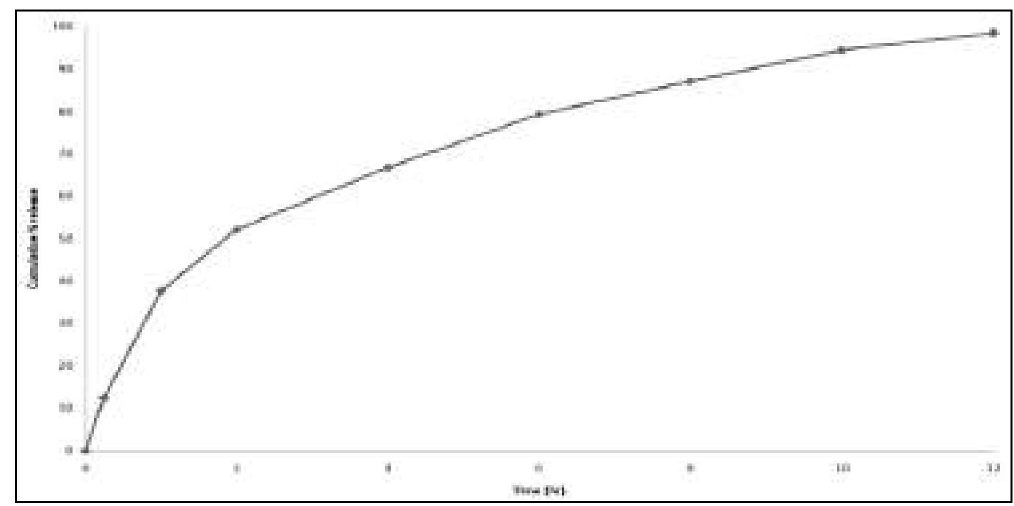

Figure 2: Dissolution profile of the optimized formulation.

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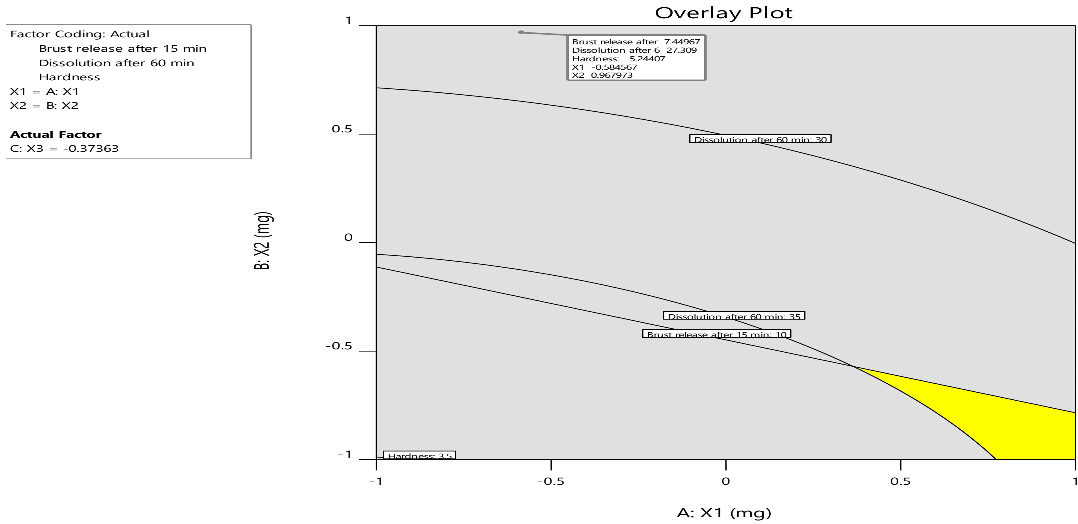

Figure 3: Overlay plot of optimized batch of formulation.

Validation of RSM result:

For all of the checkpoint formulations, the results of the physical evaluation and tablet assay were found to be within limits. Table 6 shows the composition of optimum checkpoint formulations, their predicted and experimental values of all the response variables, and the percentage error in prognosis. Linear correlation plots between the actual and the predicted response variables were plotted and the residual plots, showing the scatter of the residuals versus actual values, are presented in Figure 4. For validation of RSM results, the experimental values of the responses were compared with that of the anticipated values and the prediction error was found to vary between 0.7533% and 0.9541%. The linear correlation plots drawn between the predicted and experimental values demonstrated high values of R^2 (ranging between 0.9300 - 0.9950) indicating excellent goodness of fit ($p < 0.001$) [26]. Thus the low magnitudes of error as well as the significant values of R^2 in the present investigation prove the high prognostic ability of the RSM [20].

Table 6: Composition of optimum checkpoint formulation, the predicted and experimental values of response variables and percentage prediction error

Formulation composition (X1:X2:X3)	Response variable	Experimental value	Predicted value	Percentage prediction error
7.5:10.0:37.5	Y1 (%)	6.69	6.3108	0.3791
	Y2 (%)	24.39	24.3437	0.04625
	Y3 (kg/cm2)	5.5	5.4041	0.09583
22.5:10.0:25.0	Y1 (%)	8.13	7.6995	0.4304
	Y2 (%)	28.73	28.9125	-0.1825
	Y3 (kg/cm2)	5.5	5.4666	0.03333
7.5:10.0:37.5	Y1 (%)	7.23	7.2733	-0.0433
	Y2 (%)	26.48	26.2962	0.1837
	Y3 (kg/cm2)	5.5	5.2791	0.2208
22.5:30.0:25.0	Y1 (%)	9.63	10.272	-0.642
	Y2 (%)	34.27	33.9037	0.3662
	Y3 (kg/cm2)	4	3.9041	0.09583
7.5:20.0:12.5	Y1 (%)	12.41	11.4558	0.9541
	Y2 (%)	37.61	37.6562	-0.04625
	Y3 (kg/cm2)	3.5	3.5291	-0.0291
22.5:20.0:12.5	Y1 (%)	11.87	11.677	0.1929
	Y2 (%)	37.53	37.6662	-0.1362
	Y3 (kg/cm2)	3.5	3.2791	0.2208
7.5:20.0:37.5	**Y1 (%)**	**7.16**	**7.4945**	**-0.3345**
	Y2 (%)	**26.76**	**27.1262**	**-0.36625**
	Y3 (kg/cm2)	**5**	**5.0291**	**-0.0291**
22.5:20:37.5	Y1 (%)	9.27	9.1045	0.1654
	Y2 (%)	33.41	33.09	0.32
	Y3 (kg/cm2)	3.5	3.5916	-0.0916
15.0:10.0:12.5	Y1 (%)	8.13	8.8833	-0.7533
	Y2 (%)	32.21	32.9133	-0.7033
	Y3 (kg/cm2)	4	4.4666	-0.4666

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15.0:30.0:12.5	Y1 (%)	10.71	10.4933	0.2166
	Y2 (%)	35.54	35.7237	-0.1837
	Y3 (kg/cm2)	3.5	3.6541	-0.1541
15.5:10.0:37.5	Y1 (%)	8.56	8.662	-0.10208
	Y2 (%)	30.12	30.44	-0.32
	Y3 (kg/cm2)	4	4.0916	-0.0916
15.0:30.0:37.5	Y1 (%)	6.81	6.0895	0.7204
	Y2 (%)	21.84	21.70375	0.1362
	Y3 (kg/cm2)	5.5	5.6541	-0.1541
7.5:10:12.5	Y1 (%)	10.45	10.067	0.3829
	Y2 (%)	35.63	35.4475	0.1825
	Y3 (kg/cm2)	4.5	4.7166	-0.2166
7.5:10:12.5	Y1 (%)	7.33	8.8833	-1.5533
	Y2 (%)	32.79	32.9133	-0.1233
	Y3 (kg/cm2)	4.5	4.4666	0.0333
7.5:10:12.5	Y1 (%)	8.87	8.8833	-0.0133
	Y2 (%)	33.74	32.9133	0.8266
	Y3 (kg/cm2)	5	4.4666	0.5333

* In bold case value shows optimized batch

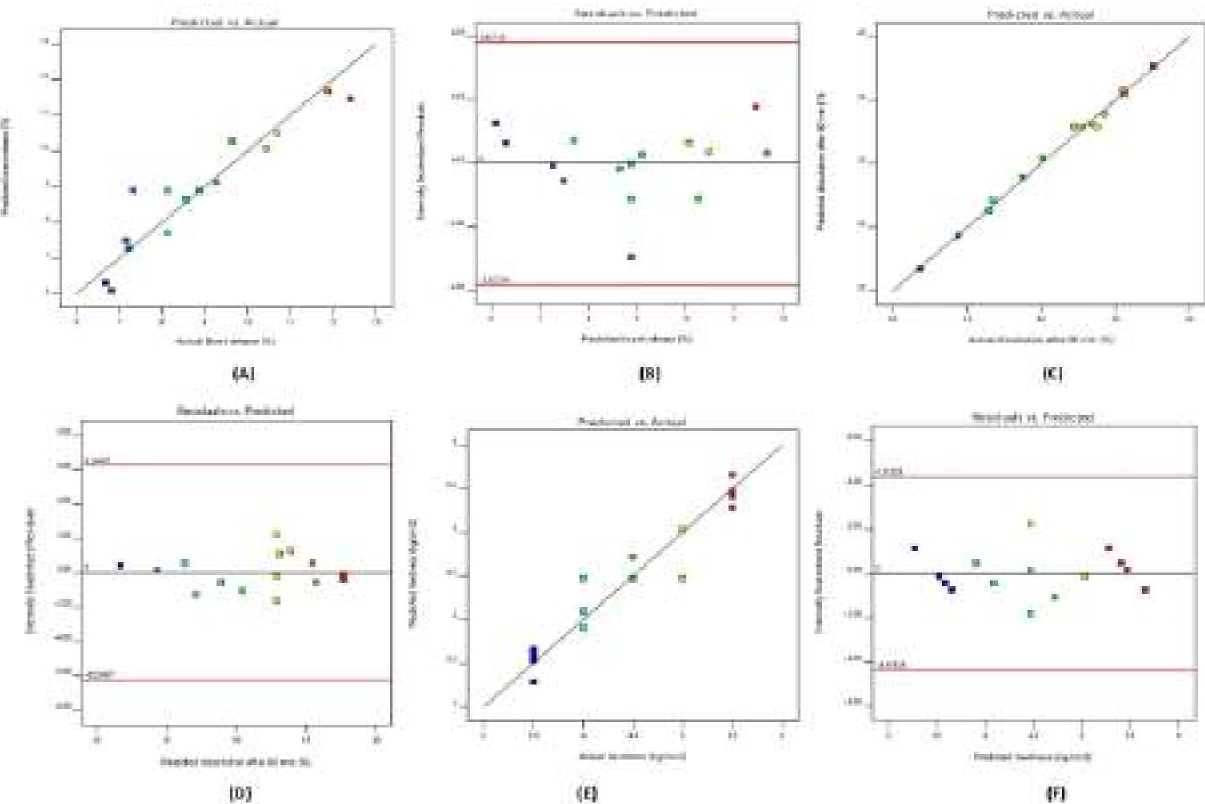

Figure 4: Linear correlation plots (A, C, E) between actual and predicted values and the corresponding residual plot (B, D, F) for various responses.

Swelling studies:

The swelling and erosion behavior of the optimized matrix tablet in 0.1N HCl and in PBS, pH 6.8, as a function of time, is shown in Figure 5. It can be observed that the hydrophilic matrix tablets underwent both swelling and erosion at the same time. The tablets achieved maximum swelling after 1 hr, which can be linked to the initial burst release of LP. Constant release can be obtained from such hydrophilic systems because of the simultaneous swelling and erosion of the matrix tablets. Constant release in such situations occurs because the increase in diffusional path length due to swelling is compensated by continuous erosion of the matrix. The cross-sectional SEM images of matrix tablets after 2 hr in acidic and 6 hr in basic media are shown in Figure 6 (A) (B). SEM study of the dissolving matrix tablets showed a uniform swelling of the matrix and further supported the fact of drug release by a diffusion process from the highly porous and swollen matrix tablets (figure 6).

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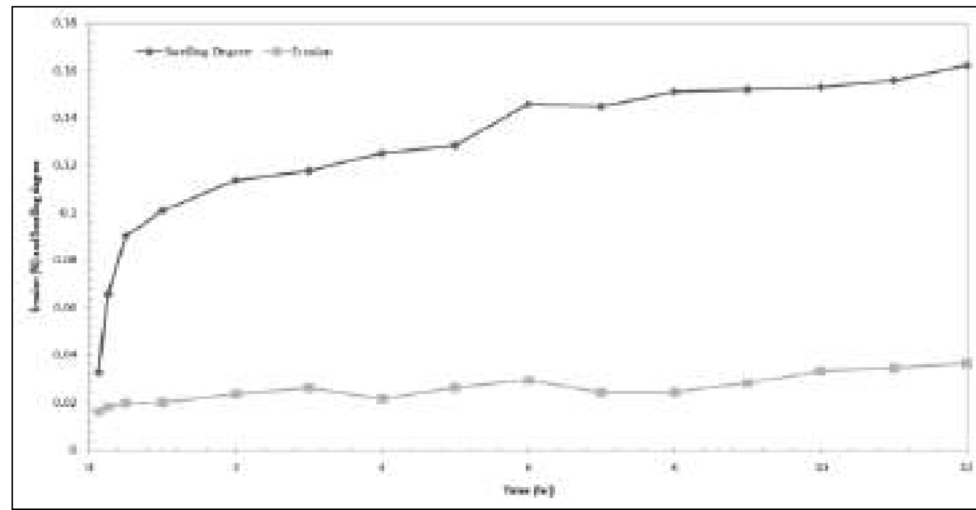

Figure 5: Erosion and swelling behavior of optimized formulation.

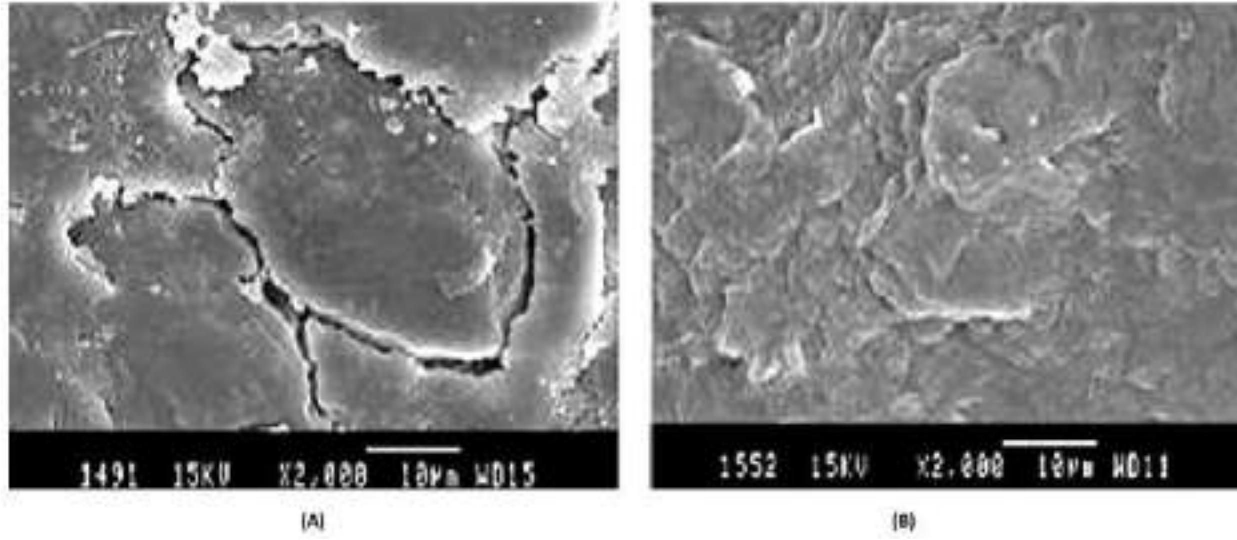

Figure 6: SEM photomicrographs showing surface topography of hydrated matrices in (A) acidic media, 2 hr (B) basic media, 6 hr.

Thermal properties:

DSC thermogram of the drug, excipients and the optimized formulation were recorded, in order to determine the thermal changes of polymers and drug before and after preparation. The characteristic endothermic peak of the drug at 255.46°C was observed in formulation also. However, the broadening of the drug peak in optimized formulation was related more to the impurities from excipients than physical interaction of the drug with the components.

Compatibility study of Losartan potassium by Fourier transform infrared (ATR-FTIR) spectroscopy:

FTIR spectra of the drug, excipients and the optimized formulation were recorded in range of 4000 – 400 cm^{-1}. LP showed some prominent and characteristic peaks at 3394 cm^{-1}, 1026 cm^{-1}, 1643 cm^{-1}, and 764 cm^{-1}, which could be assigned to stretching vibrations of O-H and C-O bond of primary alcohols, N=N stretching and C-Cl bond, respectively. In the optimized formulation, the presence of all the characteristic peaks of the LP indicates lack of any strong interaction between the drug and the excipients.

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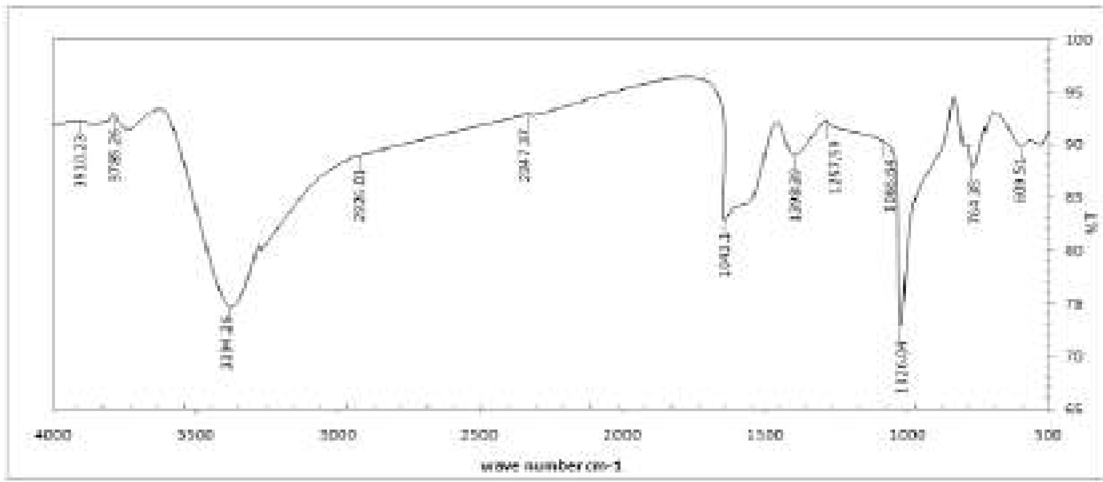

Figure 7: FTIR spectra of optimized formulation

CONCLUSION:

Release modifier polymer aminated fenugreek gum, aminated tamarind gum and aminated xanthan gum was synthesized and characterized. The synthesis polymers are characterized using ATR-FTIR, DSC and XRD studies. In this study, synthesis polymers are confirmed by ATR-FTIR study. The ATR- FTIR study of aminated fenugreek gum, aminated tamarind gum and aminated xanthan gum is confirmed by the appearance of a new peak at 3271 cm^{-1}, 1639.49 cm^{-1} and 2899.01 cm^{-1} respectively corresponding to NH2 group in the FTIR spectra of aminated fenugreek gum. Hydrophilic matrix tablets of LP with AFG, ATG and AXG were prepared and optimized using a three factor, three-level Box Behnken design. The quantitative effect of these factors at different levels on the release rate could be predicted by using polynomial equations. Linearity observed between the actual and predicted values of the response variables suggested the prognostic ability of the RSM design. The quadratic response surface methodology studied for the release rate helped in understanding the interaction effects between the combination and ratio of the three polymers. DSC and FTIR studies combined with the stability study of the optimized formulation proved the integrity of the developed hydrophilic matrix tablets. Thus, high degree of prediction obtained using RSM is quite efficient in optimizing drug delivery systems that exhibit non-linearity in responses.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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A Review: Mechanism and Role of Superdisintegrants in the Development of Mouth Dissolving Tablets

V.G.Raut[1], P.S.Nikam[2], B.P.Chaudhari[3], V.K.Redasani[4].

[1]M.pharmacy, Department of Pharmaceutics,
YSPM's Yeshoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara 415011, Maharashtra, India.
[2]M.pharmacy, [1] Department of Pharmaceutics,
YSPM's Yeshoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara 415011, Maharashtra, India.
[3]Assistant Professor, YSPM's Yeshoda Technical Campus,
Faculty of Pharmacy, Wadhe, Satara 415011, Maharashtra, India.
[4]Professor, YSPM's Yeshoda Technical Campus,
Faculty of Pharmacy, Wadhe, Satara 415011, Maharashtra, India.

Abstract : Because of their ease of administration and patient compliance, mouth dissolving tablets have become more common among strong dosage types. They outperform traditional tablets in terms of efficiency. It aids in the enhancement of oral bioavailability. Waterless administration and quick onset of operation are two major advantages of mouth dissolving tablets. For any solid dosage type, disintegration is a critical phase. Superdisintegrants are a class of younger agents that have been produced in recent years. Superdisintegrants come in a variety of forms, including normal, synthetic, and co-processed. The aim of this article is to discuss the different types of superdisintegrants and their mechanisms in mouth dissolving tablets.
Keywords -Disintegration, Superdisintegrants, Mouth dissolving, Classification, Mechanism.

1.Introduction:

In an aqueous atmosphere, superdisintegrants are agents applied to tablet and certain encapsulated formulations to facilitate the breakdown of tablet and capsule "slugs" into smaller fragments, thus expanding the available surface area and facilitating a more rapid release of the medication material. They help the tablet matrix to absorb moisture and disperse.[1-3]

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Mouth dissolving tablets are new medication delivery devices with fast disintegration capabilities that have recently gained prominence by addressing the drawbacks of traditional tablets. It is a solid unit dosage type containing active agent that disintegrates rapidly as it comes into contact with saliva without the use of water or chewing.[4]Disintegration is a crucial stage in the operation of any solid unit dosage type, such as tablets or capsules. Disintegrating agents are used in the solid dose formulations in this case. Fast disintegration is essential for quicker drug release and action in mouth dissolving tablets, so superdisintegrants are added to help with faster disintegration. They're used at a lower concentration of 1-10% by weight of the overall weight of the dosage units.[5] Different forms of superdisintegrants are available, and they are used in mouth dissolving tablet formulations depending on their source and method of action. Tablet disintegration is influenced by a number of superdisintegrant causes, including.[6]

Percentage of disintegrants present in the formulation.

a) Proportion of superdisintegrants used.
b) Compatibility with other excipients.
c) Method of addition of superdisintegrant.
d) Presence of surfactants.
e) Nature of drug substance added.
f) Hardness of the tablets.
g) Method of mixing of addition.[7,8]

Because disintegration is so important in tablet dissolution before the active drug substance is finally released from the tablet structure into the body, disintegrant properties (e.g., disintegration time [DT] and the ratio of crushing strength-friability to disintegration time [CSFR/DT]) are influenced to a large extent by the type, concentration, and efficiency of disintegrants.[9]

Advantages of superdisintegrant:

- Should be seen at low concentrations.
- Less focus is needed.
- Intragranularly, it's more powerful.
- It is biodegradable.
- Wetting has a remarkable ability to cause accelerated disintegration.
- There are no lumps formed during disintegration.
- It's safe to use with common medicinal agents and excipients.
- Has a lower impact on compressibility and flow capacity so it doesn't cling to the punches and dyes.
- Some are anionic, and cationic drugs can induce some in vitro binding.[10,11,12]

Disadvantages of Superdisintegrants:

- More susceptible and hygroscopic in nature;
- Moisture sensitivity causes instability;
- Expensive.
- It's time-consuming and delicate.[13]

Ideal properties of superdisintegrants:

- It can disintegrate quickly, have a low water solubility, and have excellent moulding and flow properties.
- The particle size, hydration power, and compressibility index should all be fine.
- It should be compatible with the other excipients and have tableting properties that are desirable.
- It does not form complexes with the medications, be nontoxic, and have a pleasant mouth feel.
- Effective at low concentrations and can disintegrate more efficiently.
- The tablets should be compactable and less friable.[12,14,15,16]

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Selection of superdisintegrants:

Superdisintegrant must follow those conditions in addition to its swelling properties when it is used as an excipient in the tablet formulation. The tablet disintegrant's requirements should be well specified. The perfect disintegrant should possess the following characteristics:

- Poor solubility.
- No tendency to form complexes with the drugs.
- Poor gel formation.
- Good moulding.
- Good hydration capacity.
- Good flow property.
- Good mouthfeel.
- Effective in less quantity.
- Particle size should be small.
- Should be non-toxic.
- It should compatible with other excipients and drug.[15,16,17,18]

2. Superdisintegrants:

To enhance disintegration processes, new materials known as "superdisintegrants" have recently been created.[19,20] Another type of super-absorbing substance with custom-made swelling qualities is superdisintegrants. These materials are designed to swell quickly rather than absorb large volumes of water or aqueous fluids. Superdisintegrants are used to make disintegrable solid dose forms more structurally sound. They are physically scattered throughout the matrix of the dosage form, and when exposed to a moist environment, they expand.

One gram of superdisintegrant absorbs 10-40 g of water or aqueous media on average. Following absorption, swelling pressure and isotropic swelling of the superdisintegrants particles generate stress concentrated zones with a gradient of mechanical characteristics, causing the entire structure to disintegrate, as seen in fig.1.[15]

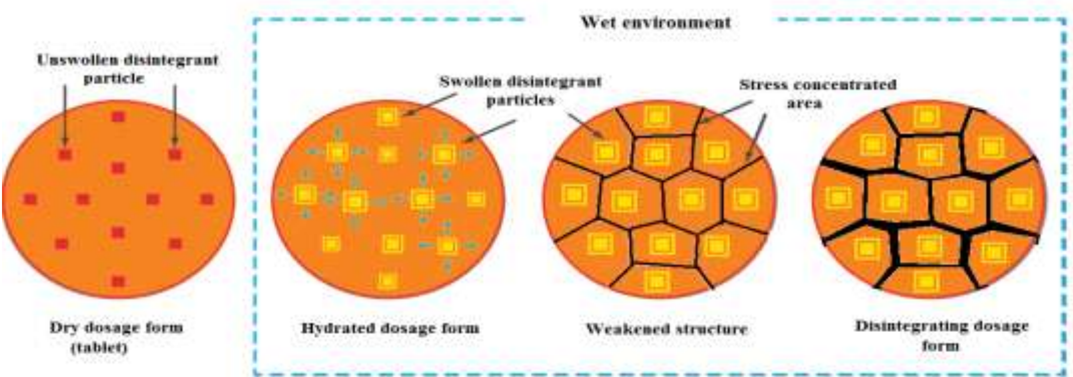

Fig.1: disintegration mechanism of superdisintegrant materials

2.1 Method of Incorporation:

The incorporation of superdisintegrants in the dosage forms are mainly of three types.

Intragranular or during granulation-

The superdisintegrants are mixed with other powders and then granulated in this procedure. Superdisintegrants are thereby absorbed into the granules.

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Advantage-

Easy to add and suitable for direct compression method.[21,22]

Extragranular or before compression-

In this process, the superdisintegrants are mixed with prepared granules before compression.

Advantage-

Suitable for wet granulation process.[21,22]

Incorporation of superdisintegrants at intra- and extra-granulation step:

A portion of the suyperdisintegrants is added to intragranular and a portion to extragranular in this process. In comparison to Type I and Type II, this approach typically yields superior results and more thorough disintegration.[23]

Advantage-

This method is more effective and provides immediate tablet disintegration.[21,22]

2.2 Mechanism of superdisintegrants:[19,24,25,26,27]

The mechanism for breaking the tablets into small pieces and producing a homogeneous suspension is as follows:

1) Swelling
2) Porosity and capillary action(Wicking)
3) Heat of wetting
4) Chemical reaction(Acid-Base reaction)
5) Particle repulsive forces
6) Deformation recovery
7) Enzymatic reaction
8) Combination action(Swelling and wicking)

2.2.1 Swelling

Tablet disintegration is most commonly caused by swelling in both natural and manufactured superdisintegrantas. When the tablet comes into contact with a suitable medium, the first stage in this mechanism is water penetration, followed by swelling of the disintegrant particle, which leads to the generation of swelling force, resulting in tablet disintegration as illustrated in fig.2.

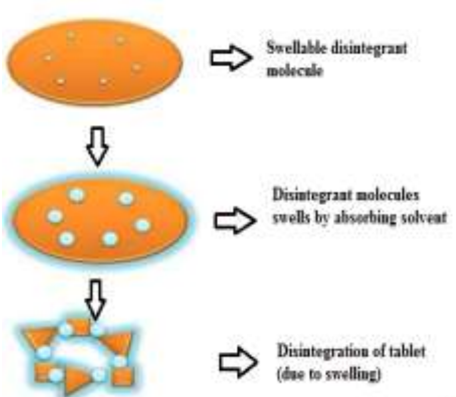

Fig.2: disintegration of tablets by swelling mechanism

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2.2.2 Porosity and capillary action(Wicking)

Porosity and capillary action are thought to be responsible for the disintegration action of effective disintegrants that do not swell. Tablet porosity creates routes for liquids to penetrate the tablet. When we immerse the tablet in an appropriate aqueous medium, the medium enters the tablet and replaces the air adsorbed on the particles, weakening the intermolecular link and causing the tablet to disintegrate into tiny particles. The hydrophilicity of the drug/excipient as well as tableting circumstances influence water absorption.Maintenance of a porous structure and low interfacial tension towards aqueous fluid is required for these types of disintegrants, which aids in disintegration by producing a hydrophilic network surrounding the drug particles, as seen in fig.3.

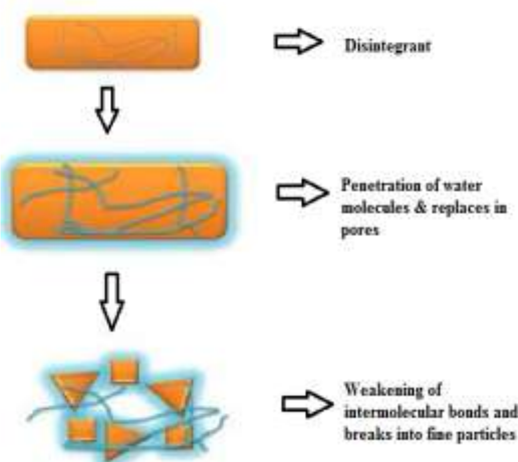

Fig.3: disintegration of tablet by wicking mechanism

2.2.3 Heat of wetting

This method can be used with any disintegrant that has an exothermic feature. When these disintegrants come into touch with appropriate media and get moist, capillary air expansion causes localised stress, resulting in tablet disintegration.[28]

2.2.4 Chemical reaction (Acid-Base reaction)

Due to the interaction of tartaric acid and citric acid with alkali metal carbonates or bicarbonates in the presence of water, the tablet is swiftly broken apart by internal CO2 release in water. The pressure within the tablet causes the tablet to dissolve.

2.2.5 Particle repulsive forces

This approach, which is based on Guyot-particle Hermann's repulsive theory, generates tablet breakdown by using non-swellable disintegrant particles. Tablet disintegration is caused by electrostatic repulsion between particles, which necessitates the use of water. Researchers discovered that wicking is secondary to repulsion.“Tablet in contact with appropriate medium, water enters into the tablet through hydrophilic pores, resulting to the production of a continuous starch-like network that assists in the transfer of water from one particle to another particle and causes hydrostatic pressure,” according to Guyot-Hermann repulsion theory. As a result, hydrogen bonds and other forces that hold tablet particles together are broken, as seen in fig.4.[29]

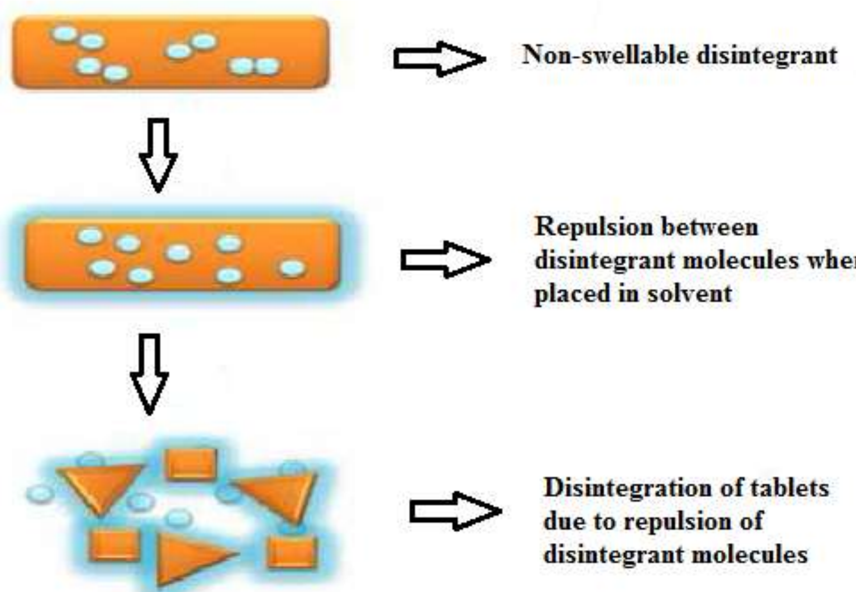

Fig.4: disintegration of tablets by repulsion mechanism

2.2.6 Deformation recovery

Starch grains are supposed to be "elastic" in nature, which means that if they are distorted under pressure, they will revert to their original shape once the pressure is released. However, because to the compression forces used in tableting, these grains are thought to remain permanently damaged and are described as "energy rich," with the energy released when exposed to water. In other words, the potential of "energy rich" starch grains to expand is greater than that of starch grains that have not been distorted under pressure. The activity of most disintegrants is thought to be the result of many mechanisms. Inter-relationships between these fundamental mechanisms are more likely to be the cause.

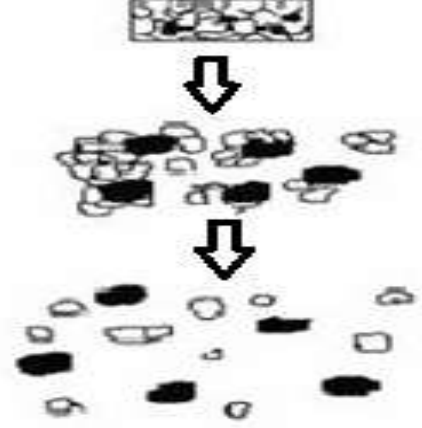

Fig.5: disintegration of tablets by deformation mechanism

2.2.7 Enzymatic reaction

Our bodies include enzymes that function as disintegrators by reducing the binder's capacity to bind. Swelling causes pressure to be applied in the outer direction, causing the tablet to rupture, or fast water absorption creates a massive rise in the volume of granules, promoting disintegration. One body enzymes which help in disintegration of tablets are given in the table 1.

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Table 1: examples of enzymes

S. No.	Enzymes
1	Amylase
2	Protease
3	Cellulase
4	Invertase

2.2.8 Combination action

The swelling and wicking mechanisms of the disintegrant induce the pill to break down.

Example: Crospovidone

2.3 Classification of superdisintegrant

a) Natural superdisintegrant
b) Synthetic superdisintegrant
c) Co-processed superdisintegrant

2.3.1 Natural superdisintegrant

Advantages

- Low cost compared to synthetic and renewable sources.
- Eco-friendly and bio-acceptable.
- Locally available.

2.3.2 Synthetic superdisintegrant

Advantages

- More effective intragranually.
- When compared to starch, it is effective at low concentrations.
- Have a negligible impact on compressibility.
- Have a minor impact on the capacity to flow.

2.3.3 Co-processed superdisintegrant

Excipient granulates are formed by co-processing excipients, which have better qualities than physical mixes of components or individual components. The procedure is used in order to achieve a synergistic change in the particular unwanted trait.

Table 2: name and mechanism of natural superdisintegrants[30,31,32]

S. No.	Name of superdisintegrant	Mechanism
1	Gaur gum	Swelling
2	Xanthum gum	Swelling property
3	Gellan gum	Swelling
4	Loctus bean gum	Swelling and capillary action
5	Agar and treated agar	High strength gelling property
6	Chitin and chitosan	Swelling
7	Mucilage of Lepidus sativum	Swelling
8	Mango peel pectin	Swelling and good solubility
9	Isapghula husk	Swelling
10	Hibiscus rosasinesislinn	Swelling
11	Soy polysaccharide	Swelling
12	Fenugreek seed mucilage	Swelling

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Table 3: name and mechanism of superdisintegrants[26,33]

S. No.	Name of superdisintegrant	Mechanism
1	Ion exchange resins	Swelling
2	Chitin and Chitosan	Swelling
3	Crospovidone	Combination of swelling and wicking
4	Croscarmellose Sodium	Swelling and wicking within 10 sec.
5	Calcium silicate	Wicking action
6	CroslinkedAlginic acid	Rapid swelling or wicking
7	Sodium starch	Absorb water quickly
8	MCC and L-HPC	-

Table 4: list of co-processed superdisintegrants

S. No.	Co-processed superdisintegrants
1	Pan Excea MH300G
2	Starlac
3	Ludipress
4	Starcap 1500
5	Ran-Explo-S
6	Ran-Explo-C
7	Ludiflast

3. Conclusion

In the creation of mouth-dissolving tablets, superdisintegrants play a significant role. In an aqueous environment, superdisintegrants aid in the breakage of the tablet into smaller fragments. Superdisintegrants have been examined in terms of selection criteria, benefits, drawbacks, ideal qualities, technique, mechanism, and categorization. The approach of adding superdisintegrants via direct compression has gained appeal among researchers. Mouth dissolving tablet formulations are less complicated than other patented methods due to their simplicity of availability and compactness.

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FORMULATION AND EVALUATION OF ANTIFUNGAL MICROEMULSION BASED GEL FOR TOPICAL DRUG DELIVERY USING MILLETIA PINNATA

Bhagyashri Gadave[1]*, Atish Velhal[2] and Dr. Vivekkumar Redasani[3]

[1]Department of Pharmaceutics, YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara 415011, Maharashtra, India.

[2]Assistant Professor, YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara 415011, Maharashtra, India.

[3]Principal, YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara 415011, Maharashtra, India.

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***Corresponding Author**

Bhagyashri Gadave

Department of Pharmaceutics, YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara 415011, Maharashtra, India.

ABSTRACT

The goal of this study was to develop and test a topical gel containing an Itraconazole microemulsion (ITZ). A preformulation research was conducted before the formulation of Itraconazole microemulsion. To determine the maximal solubility of ITZ in oils, surfactants and co-surfactants were tested to determine excipient potential. In order to microemulsion region, with Karanj oil as the oil phase, Tween 80 as the surfactant, and Isopropyl alcohol (IPA) as the co-surfactant, a pseudoternary phase diagram was created. The optimized ME of ITZ was characterized by its qualitative & Quantitative test & incorporated into polymeric gels of Carbopol (CBP), Xanthan gum, Carbopol 934, Carboxymethyl cellulose (CMC), Carboxymethyl -Tamrind gum (CMTG). ME evaluated by % transmittance, Viscosity, pH, particle size, zeta potential, Physical appearance, Drug content, pH, spreadability, viscosity, In -vitro release. Stable ME was obtained when Karanj oil was taken as oil phase, Tween 80 as surfactant & IPA as co-surfactant at the weight ratio of 5:45:50. The optimized ME based gel shows pH range 6.0- 6.34, Spreadability in the range of 0.56-1.06gm.cm/sec. The viscosity study indicated pseudoplastic behavior of all ME based gel formulations. Amongst the studied ME gels CBP: CMTG containing gels showed maximum drug release at the end of 6h. The prepared MEG show better release profile than marketed preparation.

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KEYWORDS: Itraconazole, Microemulsion based gel, Pseudoternary Phase Diagram, Topical drug delivery.

INTRODUCTION

Approximately two-thirds of the world's population is infected with a common fungal illness.[1] Fungal infection is a frequent infection that affects two-thirds of the world's population. In recent years, the prevalence of fungal infections caused by fungi including Candida, Aspergillus, and Cryptococcus has increased. Skin diseases caused by fungi are known as mycoses. Candida skin infections can affect practically any part of the body, but they're most common in intertriginous areas, where two skin patches rub or touch.[2,3]

Itraconazole (ITZ) is a triazole antifungal with a wide range of activity. It's a medication from the BCS class II. Bioavailability of Itraconazole in conventional dose formulations was around 15-20%. It has a 6-hour biological half-life. Constipation, abdominal pain, headache, and, in rare cases, heart failure have all been reported as side effects of ITZ. The fact that ITZ is contraindicated in patients with renal and/or hepatic impairment is also a drawback.[4,5]

Topical treatments, such as creams and ointments, are sticky and need rubbing, which can make patients uncomfortable. As a result of their numerous advantages over other semisolid preparations, gels have gained prominence in both the pharmaceutical and cosmetic fields.[6] Gels are characterised as a semi-rigid system in which the dispersion medium's moment is limited by interlacing three-dimensional networks of particles. They are non-invasive and patient-friendly, are less greasy, and can be easily removed from the skin. They're also affordable, have a localised action with little side effects, boost medicine absorption, reduce dose frequency, and stabilise drug distribution patterns.[6,7] Despite the many benefits of gels, one important drawback is the delivery of hydrophobic medicines. As a result, a microemulsion-based approach is being employed to break through this barrier, allowing even a hydrophobic medicinal moiety to benefit from the special features of gel.[8]

Microemulsions (MEs) have gained in popularity and attention in recent years due to their unique properties. Industrial laboratories, as well as academic researchers and those working in the pharmaceutical industry, have shown an interest in these compounds, which has led to their use in a variety of administration methods. The stable MEs are simple to make and can improve the solubilizing efficacy of both hydrophilic and lipophilic pharmaceuticals, hence increasing drug permeability.[9] ME's low viscosity, on the other hand, makes it difficult to

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apply to the skin and reduces patient compliance.[10] When compared to solution, gel, or formulations, MEs or ME gels dramatically improve medication absorption. Natural polymers are cost-effective in distribution systems because they are readily available. They're also biodegradable, biocompatible, and easily accepted by regulatory bodies.[11]

Polymers including carbopol (CBP), hydroxypropyl methylcellulose (HPMC), carboxymethyl-tamrind gum (CMTG), carboxymethyl cellulose (CMC), and in the creation of ME gels, natural polymers such as xanthan gum (XG) have been characterized.[29,12-14]

Karanj oil, a non-edible semi-drying fixed oil derived from seeds of Pongamia pinnnata belonging to the Fabaceae family, is one of the natural.[9] According to the literature, Karanj oil is a therapeutic oil that is mostly used to treat itches, abscesses, and skin problems.[10] As a result, Karanj oil can be utilised as an oil phase in the formulation of microemulsion-gels for topical delivery of drugs that are weakly water soluble, potentially improving absorption and prolonging drug release.

As a result, it was proposed to develop and test ME including topical gels of CBP, XG, TG, CMTG, and CMC for better hydrophobic drug delivery. Further research was carried out to determine the viscosity and drug release of the produced gels. ITZ's gastrointestinal adverse effects may be mitigated by a recently developed ME-based gel.

2. MATERIALS AND METHODS

2.1 Materials

Aurochem Pharmaceuticals Pvt. Ltd., Palghar, provided ITZ. Loba chemie, Mumbai, provided Tween 80, isopropyl alcohol (IPA), olive oil, Tween 20, polyethylene glycol 400 (PEG400), and carboxymethyl cellulose (CMC). S.D Lab chemical centre in Mumbai provided xanthan gum and oleic acid. All additional chemicals were acquired from Loba Chemie in Mumbai and were of analytical quality.

Table 1. Formulation of microemulsion based gels.

Formulation code	F1	F2	F3	F4	F5	F6	F7	F8	F9
Gel									
Carbopol-934 (gm)	0.5	1.0	1.5	-	-	-	-	-	-
Xanthan gum (gm)	-	-	-	0.5	1.0	1.5	-	-	-
CBP:XG (1:1) (gm)	-	-	-	-	-	-	1.0	-	-
CBP:CMC (1:1) (gm)	-	-	-	-	-	-	-	1.0	-
CBP:CMTG (1:1) (gm)	-	-	-	-	-	-	-	-	1.0

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Water (ml)	100	100	100	100	100	100	100	100	100
Microemulsion									
Itraconazole (gm)	2	2	2	2	2	2	2	2	2
Karanj oil (ml)	5.41	5.41	5.41	5.41	5.41	5.41	5.41	5.41	5.41
Tween-80:IPA (6:4) (ml)	45	45	45	45	45	45	45	45	45
Water (ml)	50	50	50	50	50	50	50	50	50
Methyl paraben (gm)	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Propyl paraben (gm)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

2.2 Solubility study of ITZ

The oils and excipients were chosen due to itraconazole high solubility in them. Based on the literature analysis, Karanj oil was chosen as an efficient excipient for micro- emulsion formation.

The solubility of itraconazole in several oils (Karanj oil, Olive oil, Oleic acid) was studied to determine the best oil for usage as the oil phase in microemulsion. Itraconazole solubility in several surfactants (Tween-20 and Tween-80) and cosurfactants (Isopropyl alcohol, propylene glycol PEG-200, PEG-400) was also investigated. In stoppered vials (capacity 10mL), an excess amount of itraconazole was added to 3mL of the specified oil, surfactant, and cosurfactant, and then preliminary mixing was carried out over magnetic stirrer for a few minutes. These vials were then held at 37 ± 0.5^{0}C for 72 hours in a mechanical bath shaker. After that, the equilibrated samples were centrifuged (Remi) for 15 minutes at 3000 rpm. The supernatant was collected, membrane was filtered and spectrometric sample measurements at 262nm. determined solubility after proper dilution by methanol.Each experiment was carried out three times.[15]

2.3 Construction of pseudoternary phase diagram

The difference fraction of mixed surfactant was often used in the building of a phase diagram, and the surfactant and cosurfactant optimal ratio (Km) was estimated using the microemulsion area. Km was investigated using a simple pseudoternary phase diagram. The generation of microemulsions utilising a four-component system consisting of an oil phase, a non-ionic surfactant, a cosurfactant, and purified water was investigated using pseudoternary phase diagrams (aqueous phase).

Titration of homogeneous liquid mixes of water, surfactant, and cosurfactant with oil phase at ambient temperature yielded the pseudo ternary phase diagram. Surfactant and co-surfactant were combined in a 1:9 to 9:1 ratio. The nine samples were mixed consistently and

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independently with water, and then the oil was added drop by drop to the mixture. Water content was set at 2.0 gm, and the total amount of surfactant and co-surfactant was also set at 2.0 gm. To allow for equilibration, samples were agitated by a vortex shaker during the titration. The combination was visually evaluated for transparency after the addition of an aliquot of oil, until the system became slightly hazy. The microemulsion window was discovered to exist as the area where clear and transparent formulations may be seen upon visual inspection. The water ratio was held constant, and the oil, surfactant, and cosurfactant formed the pseudoternary phase diagram.[16]

2.3 Construction of Ternary Phase Diagram

The best surfactant and cosurfactant weight ratio (Km) was chosen. The contents of mixed surfactant and oil in the mixtures varied from 9:1 to 1:9. A homogeneous oil surfactant–cosurfactant blend was created, where Km was fixed and the contents of mixed surfactant and oil in the mixtures varied from 9:1 to 1:9. The total amount was kept at 1.0 g. Drop by drop, purified water was added to each mixture. To allow for equilibration, samples were agitated with a magnetic stirrer during the titration. The combination was visually evaluated for clarity after an aliquot of water was added until the system became slightly cloudy.[17]

2.4 Preparation of ITZ ME

The Smix ratio with the largest microemulsion region was chosen. Oil and Smix were blended in various quantities. Itraconazole was dissolved in a mixture of oil and Smix at room temperature using magnetic stirring. Dropwise additions of double distilled water to the oily mixture were made until a clear and transparent microemulsion was formed. With mild magnetic stirring, the mixture was allowed to stabilise and reach equilibrium for 15–20 minutes. Itraconazole-containing microemulsions were then kept at room temperature.[18]

2.5 Qualitative and Quantitative tests for ME

Dilution test

The dilution test was performed by diluting 1 ml of prepared ME(s) to 100 ml and observed for clarity/turbidity/phase separation. It is confirmatory test of microemulsion to know which type of microemulsion was formed.

Centrifugation

Centrifugation test was used to evaluate physical stability of microemulsions. Microemulsions were centrifuge (Remi Laboratories, Mumbai, India) at 5000 rpm for 10 min and system was evaluated for creaming or phase separation by visual observation.[19]

pH of microemulsion

pH of microemulsion was determined by using digital pH meter (Systronics).

Transmittance (%T)

The percentage transmittance of 2ML ME(s) was checked against distilled water using UV-VIS spectrophotometer at 650 nm.

Drug Content Studies

In a 50 ml volumetric flask containing methanol, a microemulsion equivalent to 5 mg of itraconazole was placed and swirled for 30 minutes. Methanol was used to increase the volume to 50 mL. The resulting solution was further diluted by 2 ml of methanol using a membrane filter of 0.45µm.The absorbance of the solution was measured spectrophotometrically (Shimadzu UV, Japan) at 262nm.[20]

Dispersion stability studies

For 30 minutes, the formulations were centrifuged at 3500 rpm. For the heating and cooling cycle, no phase separation formulations were used (freeze thaw cycle). Six cycles were performed in a hot air oven at temperatures ranging from 4°C (refrigerator) to 45°C, with storage at each temperature for at least 48 hours. For further research, the formulations that were stable at these temperatures were chosen.[15]

Transmission electron microscopy

Transmission electron microscopy was used to examine the morphology of itraconazole microemulsion (CM200, Philips, FEI Company). One drop of diluted samples was put on film-coated copper grids, dried, and studied under the electron microscope after being negatively stained with 2 percent phosphotungstic acid (PTA).[21]

Globule size and zeta potential measurements

The globule size and zeta potential were assessed using the zetasizer nano-zs (Malvern instrument). At a temperature of 25°C, the experiment was carried out. A 1ml sample was diluted with double distilled water.[22] The globule size and zeta potential were assessed using

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the zetasizer nano-zs (Malvern instrument). At a temperature of 25°C, the experiment was carried out. Double distilled water was used to dilute a sample of 1ml.[22]

2.7 Preparation of ME based gels of ITZ

Distilled water was used to make blank gels of various polymers. In a nutshell, the polymer was dispersed in 100 mL distilled water and blended for 60 minutes using a mechanical mixer (Remi). For carbopol gels, triethanolamine was utilised as an alkalising agent.[23] For the ME preparation, the preservative was first thoroughly combined with a mixture of oil and Smix. The medicine, Itraconazole, was then dissolved in the aforesaid mixture at room temperature using magnetic stirring. Dropwise additions of double distilled water to the oily mixture were made until a clear and transparent microemulsion was formed. With mild magnetic stirring, the mixture was allowed to stabilise and reach equilibrium for 15–20 minutes. All itraconazole-containing microemulsions were then kept at room temperature.[18] The gels and microemulsions were combined in a 1:1 ratio.[24] The following table lists the formulation batches in detail. 1.

2.8 Characterization of ITZ containing ME based gels

Attenuated total reflectance – Fourier transform infrared spectroscopy

The infraround spectrophotometer of ITZ, ME, was utilised in order to get a reduced total reflectance-Fourier transform infraroad (ATR-FTIR) (Shimadzu, IR Affinity, Japan).The samples were delivered to the ATR compartment for analysis. At an average of 25 scans and a resolution of 4/cm, the spectra for the range 600-4000/cm were acquired.

Physical examination

Prepared ME based gel formulations were investigated for physical characteristics like colour, homogeneity and phase separation.[25]

Drug Content

Drug content of emulgel was measured by UV spectrophotometer. 1 gm of emulgel was diluted to 50 ml with methanol. 2ml of this solution was further diluted methanol. The absorbance of the solution was measured spectrophotometrically (Shimadzu UV, Japan) at 262nm.[26]

Spreadability study

1gm of itraconazole emulgel was placed in a 1 cm diameter circle pre-marked on a glass plate, which was then covered with a second glass plate to assess spreadability. The upper glass plate was permitted to rest for 5 minutes with a weight of 500 grams on it. The gel spreading was noted from the change in diameter of gel placed.[27]

Determination of pH

The pH of itraconazole emulgel was determined by using digital pH meter (Systronics), at ambient room temperature.[28] The calibration of pH meter was done with buffered solution before each use.

Rheological Studies

The viscosity of the different emulgel formulations was determined at 25°C using a cone and plate viscometer (Brookfield rheometer RS plus).[29]

In vitro drug release studies

A Franz diffusion (FD) cell was used in the in vitro drug release research (with effective diffusion area 3.14 cm2 and 25 ml cell volume). The formulation was applied to the FD cell's egg membrane, which was sandwiched between the donor and receptor compartments. As a dissolving medium, phosphate buffer pH 7.4 was utilised. A circulating water jacket kept the temperature of the cell at 37 0C. The solution was continuously stirred using a magnetic bead while the entire assembly was kept on a magnetic stirrer. As a control, a similar blank set was run at the same time. At appropriate time intervals, a sample (1 ml) was taken and replaced with equal volumes of fresh dissolving media. After proper dilutions, samples were tested for drug content using a UV visible spectrophotometer (Shimadzu UV1800). The total percentage of drug released was computed.[30]

3. RESULTS AND DISCUSSION

3.1 Solubility of ITZ

ITZ's physicochemical features indicate that it could be useful for topical medication delivery. Karanj oil (108.40±1.59) had the highest ITZ solubility among the selected oils that were examined, hence it was chosen as an oil. Tween 80 (246.62±16.08) demonstrated reasonable solubilizing capability for ITZ among the surfactants . ITZ is most soluble in the co-surfactant isopropyl alcohol (IPA) (Freely soluble).

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Table 2: Solubility of itraconazole in various oils, surfactants and co-surfactants.

	Vehicle	solubility of itraconazole (mg/ml)
Oils	oleic acid	64.02±1.32
	Karanj oil	108.40±1.59
	Olive oil	25.68±1.37
Surfactants	Tween-20	190.12±17.12
	Tween-80	246.62±16.08
Co-surfactants	Isopropyl alcohol	Freely soluble
	Propylene glycol	151.89±18.3
	PEG-200	110.58±15.52
	PEG-400	125.65±16.3

3.2 Construction of Pseudoternary Phase diagram

The pseudoternary phase diagram of oil (Karanj oil)/IPA / Tween 80/ water system were constructed as shown in Figure. The region giving clear and transparent formulation was considered as the ME window and was marked in pseudoternary phase diagram. The best weight ratio of surfactant and cosurfactant (Km) was discovered to be 6:4, thus for subsequent investigation, the best surfactant combination (Smix) comprising Tween 80 and IPA in a 6:4 ratio was blended with the highest oil (Karanj oil).

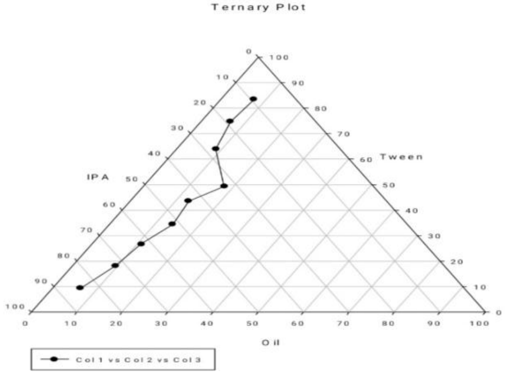

Figure 1: Pseudoternary phase diagram of the system containing Karanj oil, Tween 80, IPA and water.

3.3 Ternary Phase Diagram

The region of ME and concentration ranges of components used for formulation of ME were determined by phase studies. The effect of different surfactant /cosurfactant weight ratios on extent of stable ME region was also studied. The phase diagram of the system including oil, Smix, and water was created and is shown in fig. The microemulsion zone (ME region) in the figure is black, whereas the non-ME region is white. It is evident from the figure that tween80 and IPA could give considerable micro emulsification region (>40%).[15]

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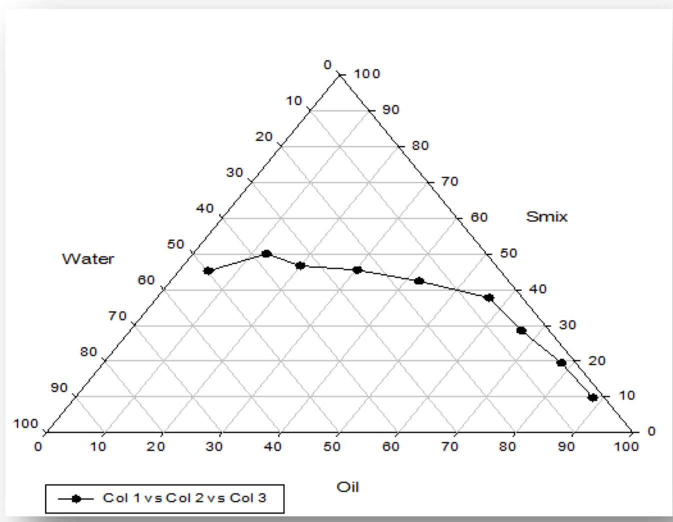

Figure 2: Phase diagram of the system containing Karanj oil, mixed surfactant and water.

3.4 Preparation of ITZ MEs

The Smix ratio with the highest ME region was chosen from the ternary phase diagram. When the weight ratios of Oil: Smix : water of 5:45:50 [M1], 10:45:45 [M2], and 10:50:45 [M3] were utilised, oil-in-water ME was generated.

3.5 Qualitative and quantitative tests of MEs

Results of qualitative and quantitave tests of all prepared MEs are given Tables.

Dilution Test

Except for formulation M1, all microemulsions generated showed phase separation and turbidity.

Centrifugation

Centrifugation test was performed to evaluate physical stability of micro-emulsions. Formulations M2 and M2 showed creaming /phase separation while other formulation was stable at centrifugation.

pH of microemulsion

The pH values of microemulsions were varied from the range 5.06 to 5.15 which was acceptable pH of skin.[31] This is an important parameter as the skin pH ranges between pH 5.0-6.5.

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Transmittance (%T)

Transmittance for all formulations are given in table and found to be in the range of 71.2 to 98.3 %. Formulation M3 shows less transmittance due to turbidity while formulation M1 shows high transmittance due to clarity.

Drug Content Studies

Dispersion stability studies

The formulations M1 stable at these temperatures were selected for further studies.

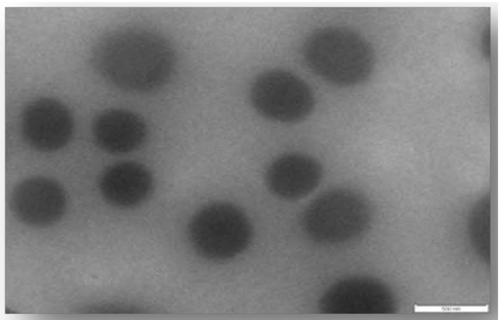
From above results the formulation M1 shows more stability than other formulations. So, M1 microemulsion was further incorporated into gelled base.

Table 3: Dilution, Centrifugation, pH, Transmittance, Drug content, Dispersion stability studies results.

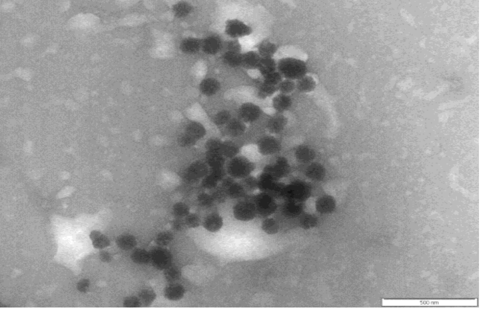
Formulation code	M1	M2	M3
Dilution test	No phase separation	Phase separation	Phase separation
Centrifugation/ creaming	No	Yes	Yes
pH	5.15	5.11	5.06
Transmittance	98.3	75.5	71.1
Drug content	99.3	98.5	95.1
Dispersion stability	Stable	Unstable	Unstable

Transmission electron microscopy

In the transmission electron microscope, the globules of optimised ME seemed to be virtually spherical in shape. In the light environment, the globule appeared dark (Fig.).The average droplet size of optimized ME was 136.4 nm. The globule size of optimized ME increases as compared optimized blank ME.



Test ME TEM



Blank ME TEM

Fig 3: Transmission electron microscopy.

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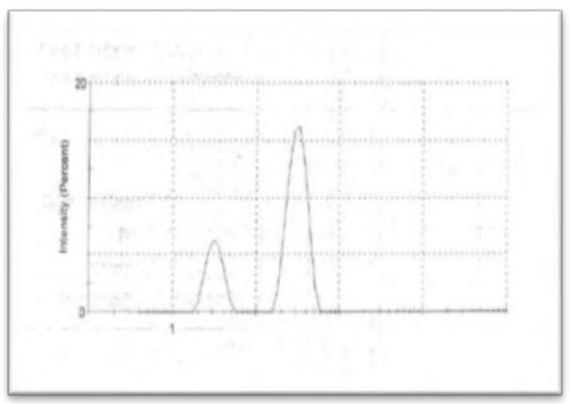
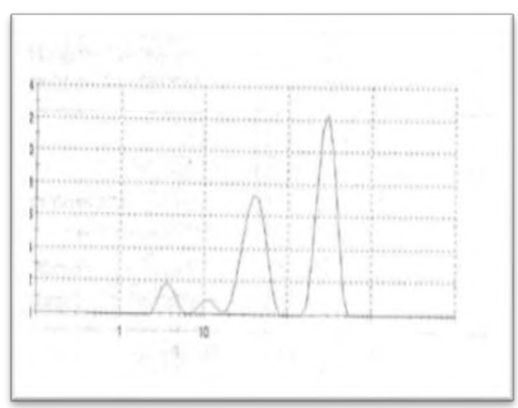
Measurement of globule size and zeta potential

Globule sizes of microemulsion were found to be 885.5dnm and 136.4dnm respectively test and blank ME formulations. The small globule size of microemulsion was due to large percent of Smix. Similarly, zeta potentials were observed to be -0.118mv and 0.00365mv respectively test and blank ME formulations.

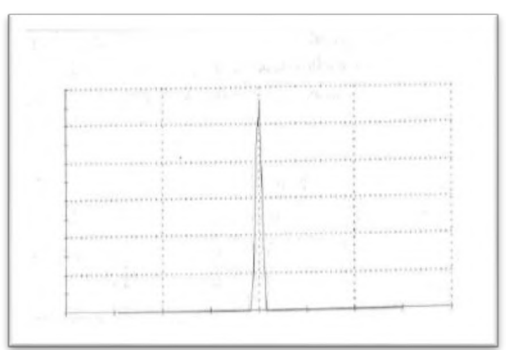


Itraconazole unloaded size distribution

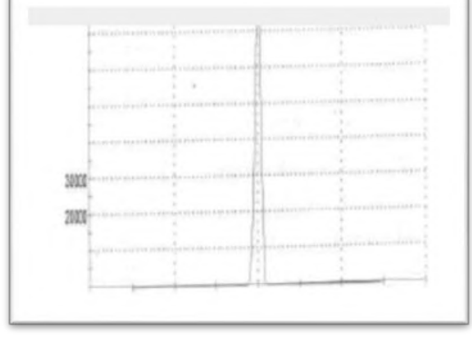


Itraconazole loaded size distribution

Figure 4: Globule size distribution.



Itraconazole unloaded zeta potential



Itraconazole loaded zeta potential

Figure 5: Zeta potential.

Table 4: Zeta potential and Globule size distribution.

Zeta potential		Globule size distribution	
Itraconazole unloaded ME (blank)	**Itraconazole loaded ME (test)**	**Itraconazole unloaded ME (blank)**	**Itraconazole loaded ME (test)**
0.00365	-0.118	136.4dnm	885.5dnm

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Evaluation of ME gel

Melting Point

The melting point of itraconazole was found to be 166.2^{0}C. The reported melting point of drug was 166-170^{0}C.

FTIR Spectrum of Interoretation

Itraconazole's FTIR spectra revealed peaks at 1583.27 (C-N stretching), 1700.91 (C=O stretching), 1187.94 (C-H aromatic), 1141.65 (C-N stretching), 3440.39 (aromatic C-H stretching), 2927.41, and 2856.66 (C-N stretching) (aliphatic C-H stretching).

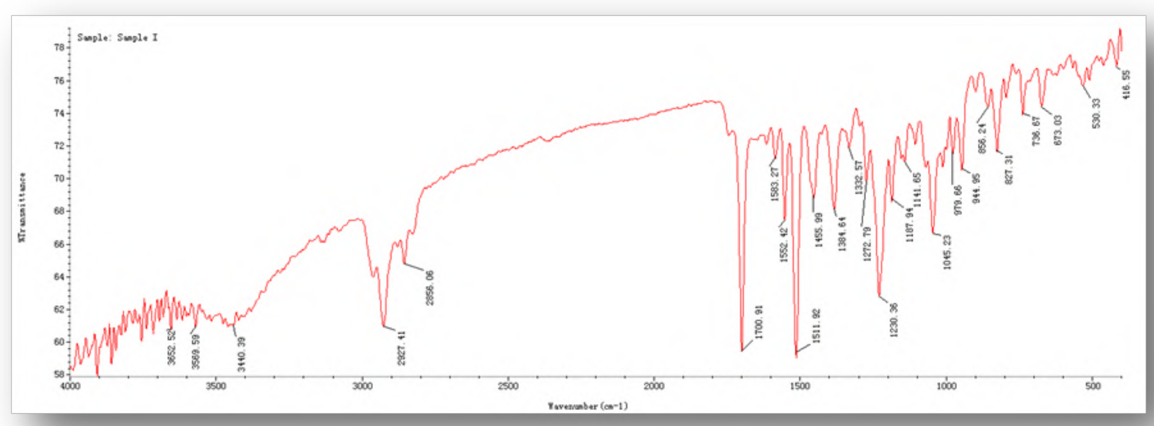

Fig 6: IR spectrum of Itraconazole.

Physical Examination

All ME-based gel formulations were white/buff thick creamy preparations with a smooth uniform texture and a glossy appearance.[33]

Drug content

Drug content was calculated using the equation, which was obtained by linear regression analysis of calibration curve of itraconazole in methanol. The drug content of all ME gel formulation was found to be 94-104%.[33]

Determination of pH

The pH values of microemulsions were varied from the range 6.09 to 6.34 which lies in the normal pH range of the skin.[34]

Spredability study

The Spreadability numbers suggested that the emulgel could be easily distributed with a minimal degree of shear. The spreadability of the gel is critical for patient compliance and aids in uniform application of the gel to the skin. A good gel will spread quickly and have a wide spreadability. ME gels prepared with low concentration of carbopol F1 belonged to fluid gel category, having more spreadability values. The stiff and semi stiff formulations were made with increasing concentrations of carbopol and xanthan gum, while the formulations F3 and F6 made with 1.5 g of carbopol were stiff and semi stiff. 1.5 g xanthan gum was classified as very stiff. The spreadability of formulations reduces as the concentration of gelling ingredient in the formulation increases.

Table 5: Spreadability studies.

Formulation Code	Drug content	pH	Spreadability gm.cm/sec
F1	102±0.14	6.1±0.69	1.06±0.2
F2	99±0.75	6.09±0.70	0.83±0.1
F3	103±0.25	6.11±0.57	0.56±0.12
F4	102±0.14	6.34±0.28	0.81±0.13
F5	100±0.15	6.19±0.35	0.76±0.17
F6	98±1.86	6.31±0.19	0.63±0.2
F7	101±0.12	6.14±0.29	0.96±0.14
F8	94±0.54	6.10±0.66	0.85±0.16
F9	99±0.6	6.12±0.48	0.96±0.10

Viscosity study

The viscosity results helped to understand the influence of various formulation parameters on consistency, spreadability and drug release. Generally consistency of formulations depends on the ratio of solid fraction to liquid fraction which produces structure.

The viscosities of ME based gels of itraconazole at low and high shear rate are given in table. Formulation containing CBP (F1-F3) exhibited high viscosity than other formulations. This is due to difference in the type of gelling agent which results in changing the structure consistency and low hygroscopicity of XG and mixture of polymers (CBP:XG), (CBP:CMC), (CBP:CMTG) (1:1) ratio as compared to CBP 934. Shear thinning was observed in all created formulations, as the viscosity was found to be reduced as the shear rate was increased (Table). Shear thinning occurs when shear is applied and the structure begins to break down when the sites of contact are disturbed and the polymeric chain aligns.Shear thinning

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behaviour is a desirable property for the topically applied preparations. Since, all prepared formulations showed pseudoplastic behaviour indicates good spreadability.

Table 6: Viscosities of ME based gels of itraconazole.

Formulation code	η* max (cP)	η** min (cP)
F1	350.47	224.36
F2	1588.24	680.96
F3	1493.45	418.39
F4	58.67	15.6
F5	1063.92	223.44
F6	1543.49	304.62
F7	1047.47	417.28
F8	868.69	332.65
F9	776.43	292.85

*Viscosity at high shear rate (100 rpm); **Viscosity at low shear rate (11.5 rpm).

In vitro drug release

All the batches of itraconazole ME gels showed drug diffusion within the range of 58.57±1.48% to 96.66±1.89% at the end of 6h.

The (CBP: CMTG) (1:1) containing gels showed maximum 96.66±1.89% drug release at the end of 6h. (CBP: CMTG) (1:1) gels exhibited higher drug release in comparison with gels formulated with CBP, XG and mixture of polymers (CBP: XG), (CBP: CMC) (1:1) ratio. As the concentration of CBP was increased in formulations (F1-F3) drug release was found to be decreased. This may be attributed to increased viscosity of carbopol gels.

Due to the difference in viscosity of the polymers, when the concentration of gelling agents in formulations increases, the diffusion of formulations reduces.

The in-vitro release of prepared formulation compared with marketed formulation (Itratrox gel 1% w/w). From the comparison it was observed that formulation F9 shows 96.66±1.89% drug release at the end of 6h and marketed Itratrox gel (1% w/w) shows 90.56±1.75% drug release at the end of 6h. From the result it was observed that ITZ ME gel of F9 batch shows more drug release compared to the marketed formulation.

Table 7: Formulation drug release percentages in Phosphate Buffer (Ph 7.4) for Formulation batches F1-F6.

Time (hr)	F1	F2	F3	F4	F5	F6
0	0.00	0.00	0.00	0.00	0.00	0.00
0.5	11.95±1.09	1.9±2.56	1.6±3.17	4.6±3.17	2.28±1.22	1.80±1.26
1	18.09±0.93	10±1.85	2.80±1.17	14.80±1.17	6.47±1.32	3.90±2.69
2	23.80±1.52	17.14±1.62	6.61±0.91	28.61±0.91	16.85±1.56	8.90±2.17
3	34.85±1.75	30.47±1.43	12.61±1.31	47.61±1.31	29.09±1.23	15.42±0.67
4	52.85±2.2	43.33±1.58	27.61±1.63	57.61±1.63	46.23±2.10	27.46±1.84
5	64.23±1.21	56.19±1.28	41.80±1.56	64.80±1.56	58.09±2.30	42.19±1.04
6	71.21±1.13	66.66±1.89	58.57±1.48	78.57±1.48	75.23±1.56	69.52±1.42

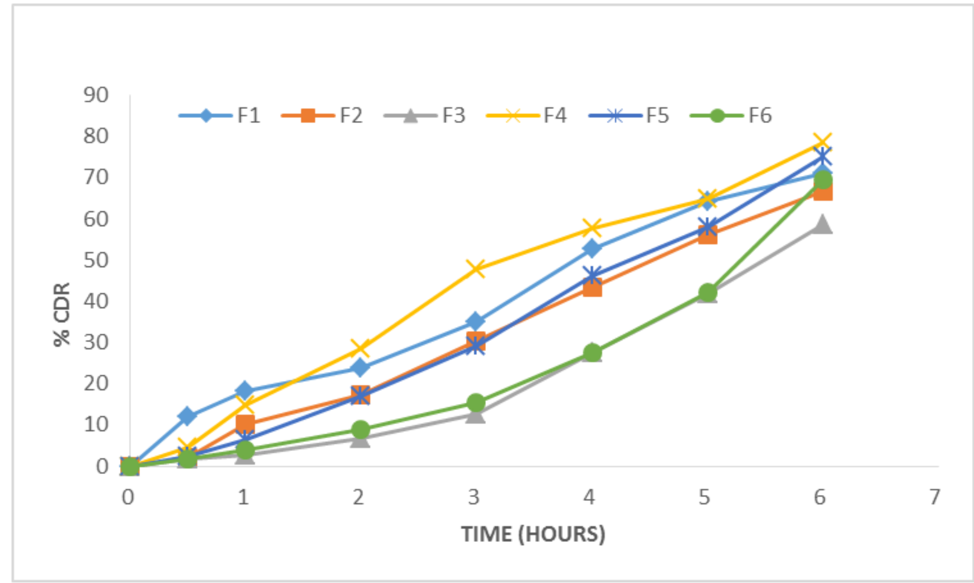

Figure 7: Formulation batch F1-F6 percentage medication release in Phosphate Buffer (Ph 7.4).

Table 8: Formulation drug release percentages in Phosphate Buffer (Ph 7.4) for Formulation batches F7-F9.

Time (hr)	F7	F8	F9	Standard
0	0.00	0.00	0.00	0.00
0.5	4.28±2.31	6.28±1.91	6.0±2.56	5.18±1.58
1	13.42±1.91	23.18±1.22	24±1.85	20.46±1.20
2	24.47±1.13	36.90±1.12	37.14±1.62	34.40±1.56
3	28.52±1.59	40.76±1.49	60.47±1.43	54.80±1.40
4	46.33±1.87	52.38±1.13	83.33±1.58	72.62±1.90
5	62.57±1.65	75.66±1.94	86.19±1.28	81.72±1.48
6	67.61±2.60	82.85±1.16	96.66±1.89	90.56±1.88

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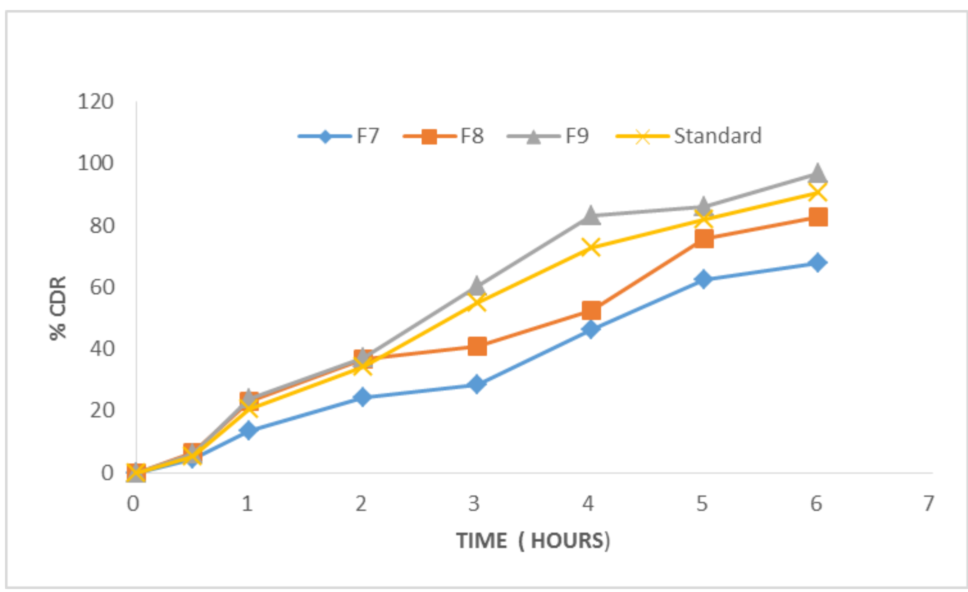

Figure 8: Percentage Drug Release of Formulations in Phosphate Buffer (Ph 7.4) for Formulation batch F7-F9.

4. CONCLUSION

ME gel produced with oil (5%), S/Cos (45%), water (50%) and (CBP: CMTG) (1:1) outperformed all other formulations in terms of overall formulation quality. Developed microemulsion system provides solubilization of hydrophobic drug, thus impart availability of itraconazole in formulation, where as globule size and zeta potential was 885.5dnm and-0.118, respectively, indicating the stability and proper formulation of microemulsion. The prepared ME gel can be considered as cost effective formulation because of reduction of topical dose of itraconazole in formulation. The F9 batch had the highest release (96.66±1.89). The prepared microemulsion gel show better release profile than marketed preparation. Furthermore, they were shown to have a better permeation and look.. It was a shear thinning system because all formulations exhibited non-Newtonian pseudoplastic behaviour. Thus, the results of this research study clearly indicated a promising potential of the itraconazole ME gel as an alternative to the conventional dosage forms.So itraconazole ME gel can be used as an anti- fungal agent for topical drug delivery.

5. ACKNOWLEDGEMENTS

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6. CONFLICT OF INTEREST

All authors approve the final manuscript and declare that there are no conflict of interests.

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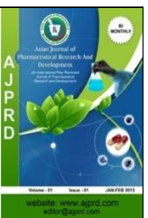

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Research Article

Evaluation of Antiepileptic Activity of Ficusracemosa in Chemicals Induced Epilepsy in Mice

Prajakta J. Chavan *, Atish B. Velhal.Vithal J Chawre, Vivek K. Redasani

Department of Pharmacology ,YSPM's Yashoda Technical Campus Wadhe ,Satara , India.

A B S T R A C T

Objective: To Evaluvate of Antiepileptic Acivity of Ficus Racemosa Extract Against Chemicals Induced epilepsy in mice, ficusracemosa is also used as antihyperglycemic, antiinflammatery , hepatoprotective action. **Method :** Anticonvulsant activity of three distinct dose levels of ethanolic extract of Ficus racemosa (100, 200, and 400 mg/kg) was tested in Swiss albino mice in seizures induced by Pentylenetetrazol (PTZ). Statistical analysis was carried out by one-way analysis of variance followed by Dunnett's test. **Results**: The presence of , flavonoids, were detected in the bark of Ficus racemosa. The extract dose-dependent effect in the delay of the onset of seizures and reduction in the duration of seizure. **Conclusion :** The ethanolic extract of Ficus racemosa exhibited significant and dose-dependent antiepileptic activity, which may be due to the presence of antioxidant principles like flavanoidsa nd other phytoconstituent produce protective activity against PTZ.

Keywords: Pentelynetetrazole, Anticonvulsant activity, diazepam, *Ficus racemosa* bark.

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***Address for Correspondence:**

Prajakta J. Chavan, Department of Pharmacology, YSPM's Yashoda Technical Campus Wadhe ,Satara , India.

INTRODUCTION:

Epilepsy is a chronic disorder of central nervous system, Epilepsy are disorders characterized by paroxysmal, abnormal, excessive or synchronous neuronal activity in the brain with 5-10% of the population. There are many antiepileptic agents available for treatment but associated with side-effects such as depression, ischemia, impaired cognition and motor disability. This made man to search for alternative medicine from natural source.

Medicinal plants used for the therapy of epilepsy in traditional medicine have been shown to anticonvulsant activities. which can be much cheaper and less time-consuming. Several useful medicines derived from plants have been discovered from scientific investigation of traditional claim.

Ficus racemosa is also known as F. Glomerata. Ficus racemosa has various synonyms like Udumbara (U gular, etc. It is used in treatment of burning sensation and obesity, anti-ulcer antipyretic antidiabetic diuretic, like astringent, and useful in vaginaldisorder Epilepsy is the term used for a group of disorders characterized by recurrent spontaneous seizures and involves hyperexcitable neurons. It is assumed that there is an imbalance between inhibitory GABA-mediated and excitatory glutamate-mediated neurotransmission. It is commonly associated with the brain.

The seizure activity during epilepsy decreases the antioxidant defense mechanism in the brain and increases the amount of free radicals, which further induces the oxidative stress. Free radicals (FR) can be defined as molecules or molecular fragments that contain one or more unpaired electrons. These free radicals were involved in causation of lipid peroxidation, brain edema and epilepsy.

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Material and Methods:

Drug andchemicals

The standard drugs of Diazepam were obtain form (Ranbaxy), Pentylenetetrazole obtained from (OZONE® INTERNATIONAL (INDIA) , All other chemicals used were of analytical grade.

Plant collection:

The bark of ficusracemosa plant were collected and shade dried and made in coarse powder

After collection Ficus racemosa bark were cleaned, washed to remove any dirt, dust and foreign particles. Botanical identity of plant specimen was authenticated by Dr. S. A. Mohite, Head, Department of Botany, Lal Bahadur Shastri College, Satara (MS), India. A voucher specimen of the bark has been deposited in the department for future reference .The bark were coarsely powdered and further utilized for preparation of ethanol extract.

Plant Extraction:

The ethanol extraction of bark of Ficus racemosa was carried out by soxhlet apparatus. The bark were crushed and ground to powder and placed into extractor. The ethanol was poured on powder with three cycles. After that extraction process was started and continued till appearance of solvent in syphon tube turns brown to clear. Then brown colored solvent mixture from round bottom flask was collected and evaporated with the help of rotary evaporator to get a solid residue. The residue was placed in a vacuum desiccator and was further used for the experiments.

Pharmacological Investigation:

Experimental Animals: Adult Swiss albino mice (25-30 g) were used for this study. The animals were housed at 24°C ± 2°C and relative humidity 55 ± 5 with 12:12 h light and dark cycle. They were provided food and water *ad libitum*. The experimental protocol was approved by the Institutional Animals Ethics Committee of Yashoda College of Pharmacy, Satara , Maharashtra.

Acute toxicity study:

The acute oral toxicity was performed as per the Organization for economic co- operation and development (OECD) guideline 423.[14]Acute toxicity study was performed in Swiss albino mice. The animals were grouped with three numbers in each were administered orally with the ethonolic extract of Ficus racemosa was given to animals with starting dose 300mg/kg in 0.1% CMC for first According to observations of first group, study was carried out further on next group with dose 2000 mg/kg. From obtained results it was clear that no death as well as no toxicological signs in animals so, for confirmation of safety of extract study was repeated with dose 2000mg/kg on third group. After administration of extract, animals were observed carefully for first 30 min and periodically for 24 h with special attention during first four hours. Animals were further observed daily for subsequent 14 days. Effects such as changes in skin fur, eyes and mucous membranes were observed daily. Animals were further observed for salivation, diarrhea, tremors, lethargy, convulsions, sleep, and coma. The parameters like body weight, food, and water intake were checked periodically every two days[20].

Evaluation of antiepileptic activity:

PTZ-induced convulsions in mice

Swiss mice of either sex were randomly divided in to Five different groups of six mice each. Group I received the vehicle, Group II received the standard drug, Diazepam at the dose of 5 mg/kg, i.p. Group III, IV and V received EEFR at the doses of 100, 200 and 400 mg/kg, p.o. respectively. Group I mice were administered with PTZ (80mg/kg, i.p.) 1 h after vehicle. Group

II mice received PTZ 30 min after Diazepam (5 mg/kg, i.p.). Group III,IV and V mice received different doses of plant extracts, p.o. 1 h before PTZ. Onset time as well as duration of convulsions were recorded [12].

Statistical analysis

The data were analyzed using one-way analysis of variance, followed by Dunnett's test. $P < 0.05$ was considered as sta1tistically significant. The data are expressed as mean ± standard deviation.

RESULT:

Preliminary phytochemicalinvestigation

Table 1.Shows the findings of qualitative analysis of Preliminary phytochemical screening of Ethanolic extract of ficusracemosa bark indicated the presence of steroids, triterpenoids, polyphenolics, coumarins, flavonoids and tannins, while alkaloids and saponins were absent.

Table 1. Qualitative analysis of the phytochemicals inextracts of *Ficus racemose bark*

Phytoconstituent	EEFR
Alkaloid	-
Carbohydrate	+
Protein	–
Steroid	+
Flavonoid	+
Tannin	–
Saponin	–
Lipid	+

+ indicating Positive , - indicate negative and EEFR indicate Ethanolic Extract of ficus Racemosa

PHARMACOLOGICAL INVESTIGATIONS

Acute toxicitystudy

The acute toxicity study began with a 300mg/kg starting dose. During a 14-day observation period, oral administration of a 300 mg/kg dosage of ethanol extract of *ficus racemosa bark* caused no significant toxicity.

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From above results it is clear that given dose was safe and hence further study was performed by administering 2000mg/kg dose of extract to next group of animals. There were no indicators of toxicity and mortality [Table 2.], as well as the animals' morphological characteristics and general appearance did not change. There was no salivation, diarrhoea, tremors, convulsions, lethargy or unusual behavior observed during study in treatment group. For further confirmation of results effect was checked by giving same dose (2000mg/kg) to another group of three animals and results parameters were normal. The oral LD50could be over 2000mg/kg body weight. As a result, greater dose testing of the extracts may not be necessary, and the extracts were practically non-toxic.

Table 2: Effect of *Ficus racemosa bark* extract for sign of toxicity and mortality (n = 3).

Group	Treatment	Sign of toxicity (ST/NB)	Mortality (D/S)
Normal Control	Vehicle	0/3	0/3
Aqueous extract	2000 mg/kg	0/3	0/3
Alcoholic extract	2000 mg/kg	0/3	0/3

STs = Sign of toxicity, NB = Normal behaviour, D = Died, S = Survived.

Table 3: Effects of ficus racemose bark extract dose 2000mg/kg on morphological characteristics and general appearance in mice (n=3)

Sr. No.	Response	Before	After
1.	Alertness	Normal	Normal
2.	Touch response	Normal	Normal
3.	Torch response	Normal	Normal
4.	salivation,	Normal	Normal
5.	Diarrhoea	Absent	Absent
6.	Tremors	Absent	Absent
7.	Convulsions	Absent	Absent
8.	Lethargy	Absent	Absent
9.	Skin fur	Normal	Normal
10.	Pinna reflux	Normal	Normal
11.	Corneal reflux	Present	Present
12.	Pupils	Normal	Normal
13.	Lacrimation	Normal	Normal
14.	Gripping strength	Normal	Normal
15.	Urination	Normal	Normal
16.	Hyper activity	Absent	Absent

PTZ induced Epilepsy

The average time of onset, duration of convulsions and percentages of inhibition of convulsions were presented in table. 3 EEFR treated mice not only exhibited delay in the onset time of convulsions at the doses of 100, 200 and 400 mg/kg, p.o. but also showed reduced duration of convulsions when compared with the control group mice. All the three doses of EEFR afforded significant protection in a dose-dependent manner against convulsions induced by PTZ ($P < 0.01$). Animals pretreated with EEFR at all the three doses exhibited significant antiepileptic activity and more percentage of inhibition of convulsions when compared with Diazepam treated animals.

Table 3: Anticonvulsant effect ethanolic extracts of Ficus racemosa on PTZ-induced convulsions in mice.

Experimental Group	Dose	Onset of convulsion(min)	Duration of convulsion(min)	Mortility	% Protection
Control	Vehicle	1.05.±0.12	.4.30 ± 0.20	6/6	0%
Standard (Diazepam)	5mg/kg	0.000.±0.00	0.00.±0.00**	0/6	100%
EEFR	100mg/kg	2.5.±0.17	2.9 ±0.40	4/6	33.33%
EEFR	200 mg /kg	3.1.±0.22	1.8± 0.03	3/6	50%
EEFR	400mg /kg	4.5.±0.25	1.2.± 0.004	2/6	66.66%

Value are mean ± SEM ;n= 6; ANOVA followed by Dunnets test. Where *p< 0.05 **p<0.01 and***P<0.001

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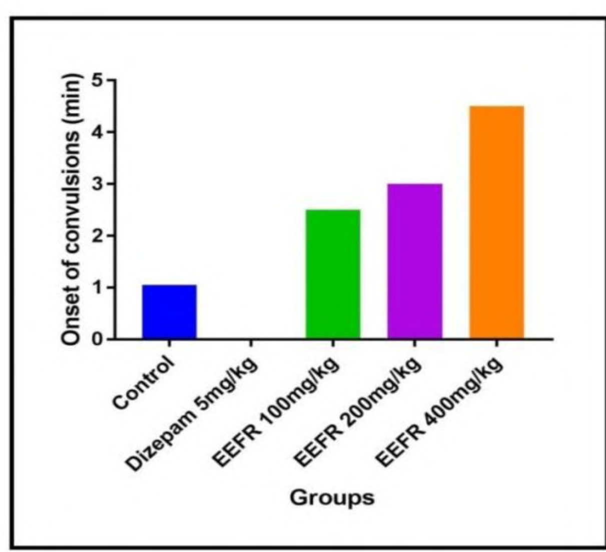
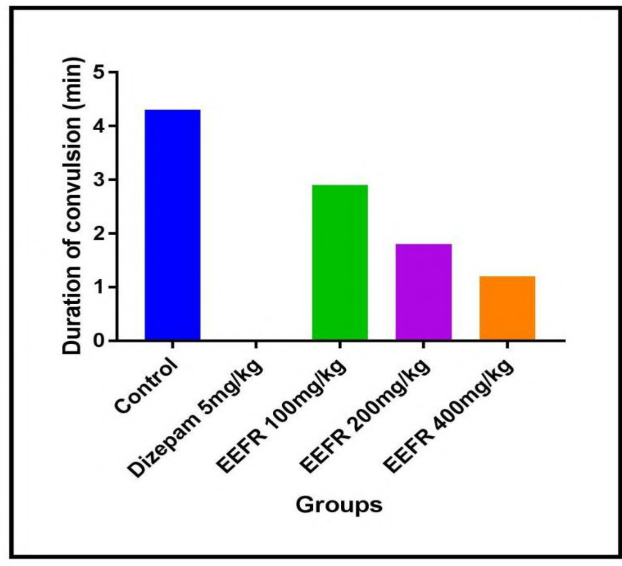


Graph 1.:Effect of EEFR on onset of convulsions in PTZ induced convulsions

Values represent mean ± SEM; n=6; Analysis was performed using one way ANOVA followed by Duneet multiple comparison test; p value less than 0.05 was considered as statistically significant. [a]p<0.05, [b]p<0.01, [c]p<0.001; [##]Data compared with control



Graph 2 : Effect of EEFR on Duration of convulsions in PTZ induced convulsions

Values represent mean ± SEM; n=6; Analysis was performed using one way ANOVA followed by Duneet multiple comparison test; p value less than 0.05 was considered as statistically significant. [a]p<0.05, [b]p<0.01, [c]p<0.001; [##]Data compared with control.

DISCUSSION:

Epilepsy is one of the chronic and most common neurological disorders. The basic and major mechanisms associated with epilepsy are increased synaptic connectivity of neurons (such as excitatory glutaminergic neurons), (weakening of potassium channels and/or inure persistent sodium channels, changes in voltage-gated ion channels), perturbance in synaptic receptors (suppressed GABAergic receptor altered nicotinic receptors), decrease in inhibitory neurotransmission (decreased GABA levels), enhanced excitatory neurotransmission (enhanced glutamate levels. [29]

In this study, anticonvulsant activity of bark extracts of Ficus racemosa PTZ-induced convulsions in Swiss albino mice.

PTZ is a potent GABA receptor antagonist, it is well known to decrease the GABA levels, and density of GABA A receptors in various parts of the brain, this leads to continuous stimulation of cortical neurons and results in convulsions similar to absence seizures is human Hence, if thought that the agents which enhance GABA levels, GABA-A receptor agonists (like diazepam), the agents behave like GARBA are thought to be useful in abolishing PTZ-induced convulsions [6].

The reports on chemical constituents of EEFR have shown the presence of antioxidant and chemopreventive principles namely, racemosic acid, bergenin, tannins, kaempferol, rutin, bergapten, psoralenes, coumarin and phenolic glycosides antioxidant used in Parkinson's ,epilepsy, Alzheimer [26][1] .

PTZ-induced convulsions in mice are a suitable model for petit mal epilepsy. PTZ is GABA antagonist. This assay has been used primarily to evaluate AED. Drugs which antagonize PTZ- induced seizures are generally useful in petit mal epilepsy. It has been indicated that PTZ- induced seizures can be prevented by drugs that reduce T-type Ca^{2+} currents, such as ethosuximide and also by drugs that enhance GABAA receptor-mediated inhibitory neurotransmission, such as benzodizepine .

It is also found that many flavonoids could act as benzodiazepine-like molecules in the central nervous system and modulate GABA-generated chloride currents in animal models of anxiety, sedation and convulsion [34][4].

The average time of onset, duration of convulsions and percentages of inhibition of convulsions were presented in EEFR treated mice not only exhibited delay in the onset time of convulsions at the doses of 100, 200 and 400 mg/kg, p.o. but also showed reduced duration of convulsions when compared with the control group mice. All the three doses of EEFR afforded significant protection in a dose-dependent manner against convulsions induced by PTZ ($P < 0.01$). Animals pretreated with EEFR at all the three doses exhibited significant antiepileptic activity and more percentage of inhibition of convulsions when compared with Diazepam treated animals. convulsion were induced the all animals by given PTZ 80 mg/kg i.p. 100% mortality was observed in control groups. Diazepam at the dose of 5 mg/kg p.o. significantly delayed onset of convulsion and decreased duration of convulsion and also delayed onset of convulsions and decreased duration of convulsions and also prototected 100% mortality rate.

CONCLUSION:

Based on the above investigations, it may be concluded that the ethanolic extract of bark of ficusracemos *a* exhibited significant antiepileptic activity. The presence of flavanoids may partially contribute the significant

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activity of EEFR by enhanced GABAergic neurotransmission which responsible for the antiepileptic effect.

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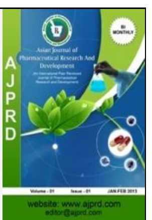

Review Article

A Review on Medicinal Plants of Natural Origin for Treatment of Polycystic Ovarian Syndrome (PCOS)

Shivani Andhalkar *[1], Vitthal Chaware [2], Vivek Redasani [3]

[1]Department of Pharmacology, YSPM's Yashoda Technical Campus Wadhe, Satara, India.
[2]Head of Department (Pharmacology), YSPM's Yashoda Technical Campus, Wadhe, Satara, India.
[3]Principal, YSPM's Yashoda Technical Campus, Wadhe, Satara, India.

A B S T R A C T

Polycystic ovarian syndrome (PCOS) related infertility is a global problem that is spreading at an alarming rate. Chronic anovulation, polycystic ovaries, and hyperandrogenism, as well as abnormal menstrual cycles, hirsutism, acne, and infertility, are all symptoms of this condition. PCOS is linked to insulin resistance and elevated levels of male hormones (androgens). Among other things, an inactive lifestyle, a lack of exercise, dietary changes, and stress are all contributing factors. Curcuma longa, Aloe barbadensis**,** Mentha piperita, Allium fistulosum Cinnamonum zeylanicum, and other plants have been shown to be effective in the treatment of PCOS. The aim of this review is to summarise the most effective medicinal plants that are used in the treatment or prevention of PCOS. Special emphasis is placed on the role of insulin resistance and the possible utility of insulin sensitizers in the treatment of PCOS.

Keywords- Polycystic ovarian syndrome (PCOS), Screening methods of pcos, Pathophysiology of pcos.

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***Address for Correspondence:**

Andhalkar Shivani, Department of Pharmacology, YSPM's Yashoda Technical Campus Wadhe, Satara, India

INTRODUCTION

Leventhal and Stein in 1935 first defined a disorder, which would ultimately become known as polycystic ovary (or ovarian) syndrome (PCOS)[1]. Polycystic ovary syndrome (PCOS), a unitization of symptoms, which affects women of child-bearing age is assumptive in epidemic proportions. A resultant of imbalance in proportion of female sex hormones, results in cysts within antral follicles of ovaries. Once multiple cysts are formed in the ovarian follicles because of the hormonal imbalance, it is characterized as PCOS. Anovulation and absence of menstrual cycle prevents fertilization, and conception in women, thus pregnancy becomes troublesome[2]. PCOS affects 6–10% of women throughout the globe. According to 1990 NIH criteria 7–12 and even more individuals. According to the broader Rotterdam criteria, which makes it one of the most common human disorders and the single most common endocrinopathy in women of reproductive age.[3] The oxidative stress (OS), that will increases in inflammation, which also been reported as possible cause of PCOS[4]. Women with PCOS has several risk factors which are associated with the development of uterine cancer including fatness, hyperinsulinemia, diabetes mellitus and abnormal uterine bleeding.[5] The frequency of depression and anxiety is higher in women with PCOS than in the general population. Mood disorders are capable of impairing quality of life, which are well-known in young adult women, concerned with fertility, and in women of all ages with respect to obesity, and clinical manifestations of excessive androgen.[8]

RISK FACTORS[6]

- Obesity
- Family history of Infertility
- Family history of PCOS
- Family history of diabetes
- Fast food diet habits
- Lack of physical exercise

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PATHOPHYSIOLOGY OF PCOS[7]

The pituitary gonadotropin is fundamental to reproductive function-its production and secretion of FSH and LH is directly stimulated by hypothalamic GnRH and it is also influenced by integrated feedback mechanisms. The initial stimulus for follicular development and also granulosa cell conversion of androgens to oestrogens by stimulating the aromatase enzymes is provided by FSH. Luteinizing Hormone(LH) characteristically known for its role in the luteal phase by promoting secretion of progesterone, also it has a vital role in the follicular phase, for inducing thecal androgen production. Women with PCOS often secrete more LH and this might result in higher theca cell androgen secretion. to maintain gonadotropin secretion pulsatile GnRH stimulation is required, but the continuous exposure of the pituitary to GnRH causes desensitisation and a suppression of gonadotropin secretion. Due to Changes in the pulsatility of GnRH alter the ratio of secretion of the two pituitary gonadotropins throughout the menstrual cycle.[7]Excessive androgen in PCOS is related with increase in abdominal fat leads to dyslipidemia and hyperinsulinemia. Thus, hyperinsulinemia reduces hepatic sex hormone-binding globulin(SHBG) to increase circulating bioactive testosterone levels.[8]

Screening Methods of PCOS

Androgen Induced PCOS Model[9]:

Hyperandrogenism is the most common symptom of PCOS. One of the etiologic hypotheses for PCOS is that early life exposure to excessive androgens leads to PCOS later in life. Increased levels of circulating androgens in the rodent affected ovarian follicular maturation and cyst development, according to a study published more than 30 years ago. Several androgens, including dehydroepiandrosterone (DHEA), testosterone propionate (TP), and 5a-dihydrotestoterone, have been used to induce an acute PCOS condition in rats through regular injection or subcutaneous implants (DHT). However, there is still some inconsistency in the reporting of endocrine hormones and ovarian histology in different models. Furthermore, some studies did not look at cardiometabolic parameters or the effects of daily androgen injections and/or treatment on physiologic indices like body weight, stress indicators, or food intake. The pathological induction of PCOS in these rodent models is transient and dependent on androgen treatment. As a result, natural reproductive/ovarian cycling happens again after androgen administration is stopped.

DHEA Induced PCOS[9]:

The first androgen to increase in the female peripubertal cycle is dehydroepiandrosterone. Nearly half of follicular synthesised T can be obtained from circulating DHEA, and 25% of PCOS patients have higher-than-normal circulating DHEA levels. Roy et al. were the first to use dehydroepiandrosterone to induce PCOS in rats. DHEA (6 mg/100 g body weight, dissolved in 0.2 mL sesame oil) is injected daily for up to 20–27 days into prepubertal rats, typically aged 22 days. Rats become acyclic and anovulatory after treatment

Ovarian Morphology: Multiple follicular cysts varying in size from 0.45 to 2.2 mm in diameter, as well as degeneration of granulosa cell layers, grow in dehydroepiandrosterone-induced rats. The ovarian tunica capsule is not thickened, and the ovarian weight of DHEA-treated rats is substantially increased.

***Endocrine hormone profile*:** DHEA-induced rats have significantly higher serum DHEA, T, E2, FSH, LH, and PRL concentrations than control rats, while no changes in plasma FSH and LH concentrations have been identified by other groups. Fasting serum glucose and insulin concentrations were higher in DHEA-induced rats, indicating cardiometabolic abnormalities.

Early DHEA-related hyperandrogenemia, anovulation, cystic ovaries, and the production of insulin glucose metabolism abnormalities can all be detected using the DHEA-induced model.

TP- *Summary*: Induced PCOS Model[10]:

Testosterone propionate (TP) can cause hyperandrogenemia in rats when given prenatally or postnatally. Furthermore, prenatal T exposure during the crucial time of foetal development has been linked to reproductive system developmental and morphological abnormalities. Pregnant rats were given a single dose injection of T on gestational day 20 or T propionate (TP) from days 16 to 19 (3 mg T daily) of pregnancy for prenatal administration. Rats were given TP at a dose of 1.25 mg/100 g body weight at 5 days of age, or daily injections of 1 mg/100 g body weight from 21 to 56 days of age.

Estrous cyclicity: T prenatally treated rats had longer and more irregular estrous periods. Estrous cyclicity was disrupted and diestrus phase was persistent in postnatally treated rats.

Ovarian morphology: In the ovaries of rats treated prenatally with T, the number of preantral and antral follicles increased, whereas the number of pre-ovulatory follicles and corpus luteum (CL) cells decreased, as opposed to control rats. In prenatal T-treated rats, cystic follicles were also discovered. Rats given T postnatally, on the other hand, had massive cystic or atretic follicles and luteinization of theca cells in the ovaries. When postnatal T treated rats were fed a high fat diet, their body weight increased while their fasting glucose levels remained unchanged.

Summary: The ovary of rats treated with postnatal T showed morphological changes that mirrored the human PCOS phenotype. Prenatal T therapy, on the other hand, increased the number of preantral and antral follicles in rats, despite the fact that cystic follicles and ovary weight were unchanged in this model, and the reported changes did not match the ovarian morphology of people with PCOS. Both prenatal and postnatal T therapy increased serum T levels. Prenatal T administration had no effect on serum E2 and P4 levels, while continuous postnatal T treatment increased E2 levels, likely due to T conversion. An increased number of kisspeptin-positive cells in the ARC of

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prenatal T-treated ewes may be linked to defects in GnRH/LH secretion feedback control 11; however, one drawback of this study is that LH levels and ovarian morphology were not examined..

DHT induced PCOS models[10]:

Since DHT is not converted to E2 by aromatase, the PCOS phenotype in DHT-treated animals can be studied without taking into account the effects of oestrogen derived from androgens.

Prenatal DHT treated models:

Mice were injected with 250 g of DHT on days 16, 17, and 18 of gestation 28 to generate prenatal DHT-treated animals, while rats were given 3 mg of DHT daily from gestational day 16 to 19. The offspring were used as PCOS models that had been prenatally treated with DHT.

Estrous cyclicity: Prenatally administered DHT caused irregular cycles in rats and mice. The mice spent more days in diestrus and fewer days in proestrus than controls, resulting in fewer litters being produced every three months.

Ovarian morphology: Prenatal DHT treatment resulted in fewer normal large, antral, preovulatory follicles and CLs, as well as more atretic cyst like follicles. In prenatal DHT treated mice, CL and antral follicle wall areas were reduced, but the number of atretic cyst like follicles and the thickness of the antral follicle theca cell layer increased.

Neuropeptides in the hypothalamus: The number of kisspeptin and NKB positive cells in the ARC of the hypothalamus increased significantly in prenatal DHT treated rats, whereas the number of kisspeptin positive cells in the AVPV did not differ from that of control animals in diestrus. The input of aminobutyric acid (GABA) to GnRH-expressing neurons was increased in mice given DHT prenatally, according to a recent study.

Metabolic features and adiposity: Prenatal DHT-treated rats and mice had body weights that were close to control animals. Prenatal DHT therapy, on the other hand, increased adipocyte region in parametrial fat and the degree compared to the control group.

DHT prenatally treated rats and mice had abnormal estrous cycles and ovarian morphology similar to PCO. In prenatal ***Summary:*** DHT-treated rodents, increased LH levels were observed, along with an up regulation of kisspeptin in the ARC. There was no discernible difference in body weight, on the other side. This phenotype is similar to PCOS, which is marked by normal body weight and increased LH secretion.

Letrozole-Induced (Aromatase Inhibitor) Rodent Model of PCOS[11] :

Abnormal follicular development and polycystic ovary may result from intraovarian androgen excess caused by circulating hyperandrogenemia or abnormal steroidogenesis. P450 aromatase, which was expressed in the placenta, ovary, and testis as well as a wide variety of human tissues, converted testosterone and androstenedione into estradiol and estrone, respectively; a decrease in the enzyme's activity could result in increased ovarian androgen production and the development of PCOS. Aromatase is the key enzyme that converts T and androstenedione into E2 and estrone, respectively. It is widely expressed in human tissues, such as placenta, ovary, and testis. Reduced aromatase activity in the ovary is one of the pathophysiologic hypotheses of PCOS development. Letrozole is a nonsteroidal aromatase inhibitor that reduces conversion of androgens to estrogens in the ovary, resulting in increased T and decreased E2 production. Excess T in the ovaries is likely to cause polycystic ovaries directly in Letrozole-treated rats. The reduction in estrogen weakens the negative feedback on LH production in the pituitary, resulting in increased LH levels, which further stimulates theca cells to secrete T. Typically, 6-week-old female rats (puberty) are administered Letrozole orally at doses of 0.1, 0.5, and 1.0 mg/kg daily for 21 days, after which they become acyclic, with histological and biochemical features of human PCOS.

Estrous cyclicity: Regular vaginal smear examinations were used to monitor estrus cycles. In the analysis, only animals with two consecutive standard 4-day periods were used. The rats and mice treated with letrozole were fully acyclic. This rat model's vaginal smears showed an excess of leukocytes, the diestrus phase's predominant cell type.

Ovarian morphology: Ovaries from control group exhibited follicles in various stages of development including secondary follicles, graffian follicles, and fresh corpora lutea. In study groups, letrozole inhibited growth of follicles in a dose-dependent manner. Small follicles could be observed in early development, in addition to follicles showing evidence of atresia, and many large cysts with virtually no granulosa cell layer or large cystic follicles with scant granulosa cells. Ovaries from the sample groups had a higher rate of subcapsular ovarian cysts and capsular thickening than the control group. together with incomplete luteinization and a dose-dependent decrease in the amount of corpora lutea In some of the research classes, there was also evidence of theca cell hyperplasia..

Summary: Acyclicity, cystic ovarian morphology, elevated serum LH levels, and higher Kiss1 mRNA expression in the posterior hypothalamus are all observed in letrozole-induced PCOS model rats compared to control rats. This model accurately reproduces the metabolic characteristics of human PCOS, including a PCO-like morphology and elevated serum LH levels, and is thus suitable for studying human PCOS. Increased KNDy neuron activity was linked to a reduction in the negative feedback effect of sex steroid hormones, as evidenced by increased Kiss1 mRNA and serum LH levels.

Medicinal plants of natural origin-

Curcuma Longa (Turmeric) ***[12]:***Curcumin is a water-insoluble polyphenolic curcuminoid derivative found in the rhizomes of Curcuma longa, an Indian spice (turmeric). Turmeric is widely used in Asian cuisine as a food additive

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and colouring agent, as well as in Indian herbal medicine. Curcumin makes up around 2–8% of all turmeric preparations. Curcumin has been shown to have a wide range of biological effects like Anti-inflammatory, antioxidant, hypoglycaemic, and antihyperlipidemic properties The study used virgin, cyclic, adult female Wistar Albino rats weighing 160–200 g. Once everyday for 21 days, followed by treatment with curcumin. Letrozole treatment resulted in abnormalities in the serum sex steroid profile, lipid profile, glucose, and glycosylated haemoglobin levels and antioxidant activity has been depleted. Whereas Curcumin was able to exert its calming effect by returning all parameters to normal and causing cysts in the ovaries to vanish. Curcumin, like Clomiphene citrate, has a number of beneficial effects in the treatment of PCOS.

Aloe barbadensis (Aloe)[13]:Aloe barbadensis Mill. (Liliaceae) is a well-known plant with such properties. Polyphenols, sterols, flavanoids, and other nutrients were analysed qualitatively and quantitatively for polyphenols, sterols, flavanoids, and other nutrients in the Aloe vera gel formulation. To induce PCOS, five-month-old Charles Foster female rats were orally fed letrozole, a non-steroidal aromatase inhibitor. The rats were then given the Aloe vera gel formulation orally.AVG treatment of PCO rats resulted in a reduction in ovary atretic cysts as compared to PCOS controls, according to histological review. By restoring ovarian steroid status and modifying main steroidogenic behaviour, aloe vera gel formulation protects against the PCOS phenotype.

Glycyrrhiza glabra (liquorice)[14]: Traditional medicine has used liquorice (Glycyrrhiza glabra of the Leguminosae family) to treat a variety of ailments. Antifungal, antiviral, antibacterial, and antihyperglycemic properties are all present in it. The most bioactive compound in liquorice is glycyrrhizic acid. Phytoestrogens found in liquorice include liquiritigenin, liquiritin, isoliquiritin, isoliquiritigenin, glabidin, and glabrene. The effects of two natural compounds derived from liquorice root on vascular tissues in vitro and in vivo were reported: glabridin, the main glabrene, and isoflavane, an isoflavene, both demonstrated estrogen-like activities. One of the bioactive compounds responsible for weight loss may be liquiritigenin, a selective oestrogen receptor ligand. Some molecules, such as glabrene and glabridin, have been shown to reduce weight in vivo. It has also been documented that treating hirsute women with a combination of spironolactone and liquorice may help with PCOS by reducing the volume depletion caused by spiranolactone and possibly increasing its anti-androgenic activity.

Mentha piperita(Peppermint) [15]:Peppermint (Mentha piperita L.) is a member of the Labiatae family that originated in the Mediterranean region and is now widely cultivated all over the world. Antioxidant, antitumor, antiallergenic, anti-inflammatory, antiviral, antibacterial, and antifungal properties are all present in peppermint. It also has anti-androgenic properties, lowering the level of free testosterone in the blood after three weeks of treatment with letrozole and peppermint. Females with PCOS had significant changes in serum testosterone, oestrogen, LH, and FSH function. Ovarian cysts with a reduced granulosa layer, atretic follicles, and a small number of corpora lutea were found in the PCOS community. Peppermint was found to have a strong potential as an alternative therapy in the treatment of PCOS, as shown by necrosis in stromal mesenchymal cells, hyperplasia of luminal epithelial cells, and necrosis in stromal mesenchymal cells.

Allium fistulosum (Onion)[16]: In Asian countries, the Welsh onion (Allium fistulosum) is well-known for its use in food and traditional medicine. For treatment, administered AF extract to letrozole-treated rats for 2 weeks. In terms of serum hormonal levels, the LH/FSH ratio and serum oestrogen levels were positively affected by AF extract therapy. FSH and LH are necessary for ovulation, and PCOS patients often have a two- to three-fold increased LH/FSH ratio, which is enough to cause ovulation disruption. The findings suggest that AF extract normalises follicular growth and ovarian cysts. In the letrozole-induced PCOS rat model, the steroid hormone-related receptors demonstrated restoration of m-RNA expression after treatment with AF extract A. fistulosum extract treatment relieved hormonal imbalance and altered ovarian function.

Linum usittassimum (Flaxseed)[17]: Flaxseed is made from Linum usittassimum (Linaceae), an omega-3 fatty acid-rich food that is also one of the best sources of dietary lignin. ALA, lignans (secoisolariciresinol diglycoside-SDG), and soluble flaxseed fibre mucilage (d-Xylose, L-Galactose, L-Rhamnose, d-galacturonic acid) are all biologically active compounds with major health benefits. Flaxseed or isolated lignan has been shown in studies to lower androgen levels while also normalising lipid levels. Lignans seem to minimise excess testosterone, which is a crucial factor in the development of PCOS. Flaxseed supplementation can help women with PCOS control androgen levels, according to a case study. The study found a substantial reduction in androgen levels. There was also a decrease in hirsutism. Flaxseed can have a significant effect on testosterone levels, as well as symptoms associated with hyperandrogenism, such as hirsutism, according to the findings. Another research looked at the impact of flax seeds on ovarian morphology in PCOS patients, finding that flax seed supplementation decreased ovarian volume, increased the amount of follicles in the ovaries, and improved menstrual cycle duration. However, hirsutism, blood sugar levels, or body weight did not improve as a result of the research.

Panax ginseng (Ginseng)[18]: Herbal medication is made from the roots of Panax ginseng (Araliaceae). It has anti-aging properties and is used as a tonic. Ginseng saponins are ginseng's active ingredient. Rb1, Rb2, Rc, Rd, Re, Ro, Ra, and minor ginsenosides make up these ginsenosides. It can be used as a dietary supplement. Estradiol valerate induced polycystic ovary in rats. The ovarian morphology was examined in this analysis. The ginseng-containing formulation is known as Kampo preparations**.** It is formulation significantly decreases the plasma LH levels

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and thereby it is effective in improving endocrine condition in the treatment of disturbances of ovulation in patients with PCOS.

Tribulus terrestris (Puncture vine)[19]: Puncture vine or Devil's eyelashes, Tribulus terrestris (Zygophyllaceae), plays an important role in traditional medicine. The herb Tribulus terrestris has been shown to help with polycystic ovarian syndrome. Tribulus terrestris extract was found to be successful in improving ovulation in rats with polycystic ovaries induced with estradiol valerate in a study. The extract treatment improved ovarian follicular development and normalised estrous cyclicity and steroidal hormone levels. Many herbalists believe that tribulus is an excellent overall ovarian stimulant and female fertility tonic for women with polycystic ovary syndrome.

Gymnema sylvestre (Gymnema)[20]: Gymnema sylvestre (Asclepiadaceae) is an Ayurvedic herb that has been used for thousands of years. It has a wide range of pharmacological effects, including anti-diabetic, hypoglycemic, and lipid-lowering properties. Saponins, especially gymnemic acids, are the active constituents in gymnema. Gymnema has been shown to have hypoglycemic properties in diabetic animal models. It keeps blood glucose levels in check. Metformin therapy is a convenient way to treat PCOS. Gymnema can thus be used to treat the root cause of insulin resistance. Gymnema is a good choice for PCOS because of its insulin-modulating properties and the added advantage of lowering the high triglycerides that come with the condition.

Punica granatum (Pomegranate)[21]: Pomegranate (Punica granatum of the Punicaceae family) is a fruit with a wide range of medicinal properties. Folic acid, vitamins (B2, C, B1), carbohydrates, pantothenic acid, and organic acids are all contained in the fruit. Unsaturated and saturated fatty acids are said to be present in the crop. In adult female rats, the effect of pomegranate extract in the control or management of PCOS was studied using a control and a PCOS community. The levels of free testosterone, serum oestrogen, and andrestandoin hormone were measured in the experimental community. Pomegranate extract seems to have a protective impact on polycystic ovarian syndrome hormonal imbalances, according to the report. The extract's phenolic compounds and phytosterols have been shown to help alleviate PCOS complications. Consumption of the extract, according to the report, decreases the complications associated with PCOS.

Symplocos racemosa (Lodh Tree)[22]: Symplocos racemosa Roxb, a member of the Symplocaceae family, is a common Ayurvedic remedy for female problems. It's also known as Lodhra, and it's used as a single medication or in multi-component formulations and preparations in Indian medicine. In a Letrozole-induced female rat model, the anti-androgenic properties of S. racemosa were investigated in the treatment of PCOS. Treatment with Symplocos racemosa resulted in substantial improvements in oestrogen, testosterone, progesterone, and ovarian tissue levels. It improves fertility and prevents ovarian cell dysfunction in PCOS patients.

Cinnamomum zeylanicum (Cinnamon)[23]: Cinnamon (Cinnamomum zeylanicum of the Lauraceae family) is an insulin potentiator. Insulin-stimulated glucose uptake and glycogen synthesis are controlled by this compound. Fasting and oral glucose tolerance test values were assessed in fifteen women with PCOS in a pilot study. In women with PCOS, the cinnamon extract increased insulin sensitivity. Cinnamon extract contains polyphenols and procyanidins, which potentiate the insulin signalling pathway, resulting in a hypoglycemic impact. Cinnamon's function as an adjunctive therapy in the treatment of PCOS was identified in this research. Cinnamon's impact on menstrual cyclicity and metabolic dysfunction in women with PCOS was studied in another research. It was a 45-woman randomised controlled trial. Oral cinnamon supplements were given. Menstrual cyclicity, luteal phase, and progesterone levels were all tracked. Cinnamon supplementation increased menstrual cyclicity and was shown to be beneficial in the treatment of polycystic ovary syndrome.

Vitex Negundo(Chaste Tree)[24]:Vitex negundo is a plant belonging to the (Linn) Verbenaceae family, genus Vitex, and species negundo. It's the five-leaved chaste flower, also known as monk's pepper. It has been documented to have anti-inflammatory, analgesic, antioxidant, antifungal, antiviral, and anti-inflammatory properties, as well as being used in gynaecological disorders. It also has anti-androgenic and estrogenic properties (linoleic acid-like estrogenic compounds). For the induction of PCOS, letrozole was given orally (p.o) for a duration of 21 days. The rats were then given extract of vitex negundo, which has positive effects on the ovary as well as effects on glucose tolerance, estrous cycle irregularities, LH: FSH ratio, steroidogenic enzymes, and cardiovascular parameters. It was able to successfully treat the rats with extract, which caused abnormalities in serum sex steroid profile, lipid profile, glucose, and estrous cycle. This may be attributed to the extract's phyto-components.

CONCLUSION:

The most common cause of menstrual irregularities and hyperandrogenism is polycystic ovary syndrome (PCOS). It is the most common cause of female infertility. Several risk factors for PCOS have been studied, including glucose intolerances, obesity, and dyslipidemia. Many treatments are currently available, but they are associated with moderate to serious side effects, and their high cost has led to a search for plant-based remedies to treat PCOS. In this study, summarize some of the most important medicinal plants for treating PCOS and helps with PCOD symptom relief and management. Hyperandrogenism, insulin sensitivity, fertility, and menstrual cyclicity are all aided by these plants.

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Analytical Method Development and Validation of Flecainide Acetate by Chromatographic and Spectrophotometric Techniques

Redasani VK[1]*, Agrawal YO[2], Jagtap MS[2], Mahajan HS[2] and Surana SJ[2]

[1]Faculty of Pharmacy, YSPMs Yashoda Technical Campus, India

[2]R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur, India

***Corresponding author:** Vivekkumar K Redasani, Faculty of Pharmacy, YSPMs Yashoda Technical Campus, Satara [India], Tel: +918421456827; Email: vivek.redasani@gmail.com

Research Article

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Abstract

The aim of the present work is to establish three simple, economical and rapid methods for the quantification of Flecainide acetate in bulk drug and in tablets formulation. This study is designed to validate and developed spectroscopic and chromatographic methods as per ICH guidelines. In Method A, area under curve (AUC) of zero-order spectrum was recorded between 277.00 and 310.00nm. Linearity was found in the concentration range of 20-120µg/ml. In Method B, zero-order spectra were derivatized into first-order and the AUC was recorded between 280.00 and 310.20 nm. The linearity was found in the conc. range of 20-120µg/ml. In Method C, separation was achieved using RP-HPLC by gradient elution using a C18 Qualisil BDS (250mm×4.5mm×5µm) column, a mobile phase consisting of water: acetonitrile (60:40 v/v) with mobile phase pH adjusted with orthophosphoric acid (pH 3.0), a flow rate of 1.0ml/min and UV detection at 296nm. The linearity was obtained in the concentration range of 10-50µg/ml. The developed methods are validated successfully across various parameters in accordance with ICH guidelines. Thus, the proposed methods can be used for routine analysis of Flecainide acetate as it does not showed any interference of excipients when estimated in pharmaceutical formulations.

Keywords: Flecainide Acetate; Spectrophotometric; Chromatographic; Area Under Curve; Derivative-Spectrophotometry; Validation; ICH Guidelines

Abbreviations: AUC: Area under Curve; FA: Flecainide Acetate; LOQ: Limit of Quantification; LOD: Limit of Detection.

Introduction

Flecainide Acetate (FA) is chemically designated as N-(2-piperidinylmethyl)-2, 5-bis (2, 2, 2–trifluoroethoxy) benzamide acetate having molecular formula: $C_{19}H_{24}F_6N_2O_5$ with molecular weight: 474.4g/mol (Figure 1) [1]. It is a white solid powder with melting point 146-152°C [2]. It is an antiarrhythmic agent (class IC) causing a decreased in intra-cardiac conduction velocity in all parts of the heart [3]. It is used for the treatment of tachyarrhythmia, atrial fibrillation, supraventricular tachycardia and ventricular tachycardia by blocking Na+ current delayed rectifier K+ current [classified as a Na+ channel blocker drug] [4]. Flecainide also has local anesthetic effects. It selectively increases anterograde and retrograde accessory pathway refractoriness. The action of Flecainide in the heart prolongs the PR interval and widens the QRS complex. The effect on the JT interval is insignificant as Flecainide does not lengthen ventricular repolarization [5]. The drug is official in the European [6], British [7] and US pharmacopeias [8]. Several chromatographic methods have been reported in the literature for the determination of FA in its bulk powder, in pharmaceutical formulations or in the presence of its enantiomers, metabolites or other

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antiarrhythmic drugs [4].

Figure 1: Chemical Structure of Flecainide Acetate.

Analysis part is an important for formulation development of any drug molecule. A suitable and validated method should be vacant for the drug delivery system for analysis of bulk drug and formulation [9]. The developed methods were validated for accuracy, precision, ruggedness, and sensitivity. Accordingly, the objective of this study was to develop and validate the simple spectrophotometric method for the estimation of FA in bulk and tablets as per ICH guidelines.

Experimental

Instrumentation

The Shimadzu double beam UV-VIS spectrophotometer with spectra manager software UV Probe 2.21 with 10mm quartz cells was used. The chromatographic separation was achieved by using Agilent system consisting photodiode array detector, C_{18} Qualisil BDS (250mm×4.5mm×5µm) column [10]. All weights were taken on an electronic balance (Model Shimadzu AUX 120).

Chemicals

The gift sample of FA was obtained from Indeus Life Sciences Pvt. Ltd, Mumbai. Analytical grade solvents and reagents were purchased from Merck specialties Pvt. Ltd. Mumbai (India). Double distilled water filtered through the membrane filter was used. Flecarite tablets each containing 100mg of active drug were purchased from the local market.

Preparation of Standard Stock Solution

The standard stock solution was prepared by dissolving 10mg of FA in the 100ml of water, sonicated and to obtain the concentration of 100µg/ml.

Method A: Area under Curve Zero Order UV Spectrophotometric Method: Concentration of 10µg/ml was prepared by diluting 1ml of standard stock solution with 10ml of water. This solution was scanned in the UV-visible range 400-200nm. FA showed the maximum absorbance at 296 nm as shown in and the AUC of the zero order spectrums was recorded between the 277.00-310.00nm (Figure 2).

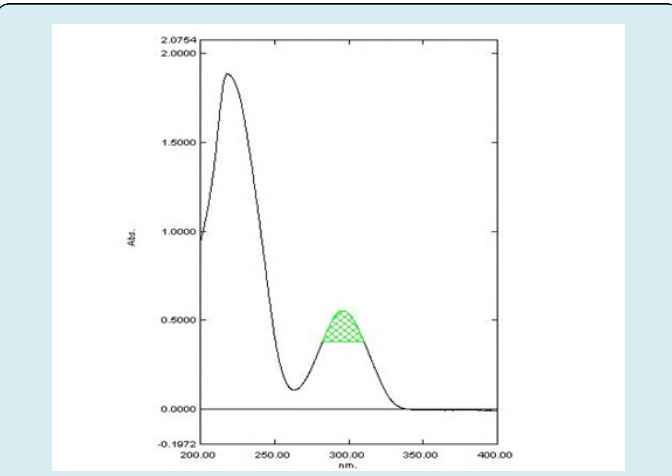

Figure 2: AUC Zero Order Derivative Spectrum of Flecainide Acetate in Water.

Method B: Area Under Curve First Order UV Spectrophotometric Method: The zero order spectrums were derivatized into first order spectrum and the AUC was recorded between 280.00-310.20nm as shown in Figure 3.

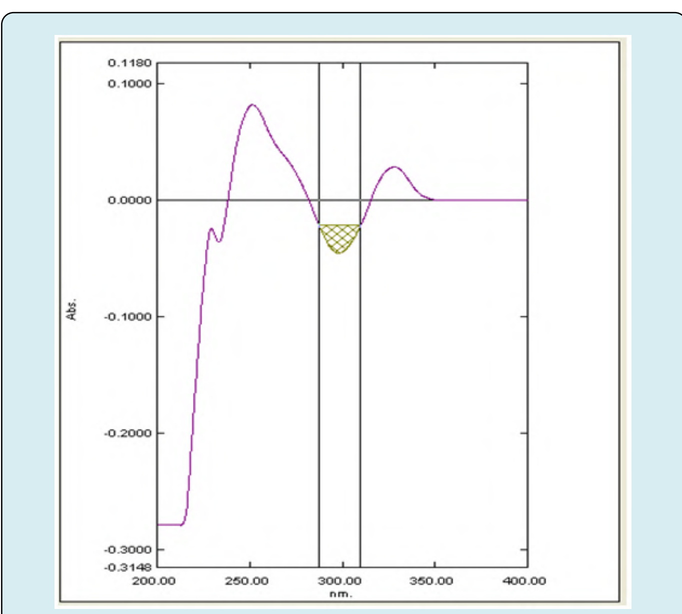

Figure 3: AUC First Order Derivative Spectrum of Flecainide Acetate in Water.

Method C: RP-HPLC Method: In Method C RP-HPLC method in which separation was achieved by gradient elution using a C_{18} Qualisil BDS (250mm×4.5mm×5µm) column, a mobile phase consisting of water: acetonitrile (60:40 v/v) with mobile phase pH adjusted with orthophosphoric acid (pH 3.0), a flow rate of 1.0ml/min and UV detection at 296nm. The linearity was obtained in the conc. range of 10-50µg/ml. The retention time of Flecainide acetate was 9.3min (Figure 4).

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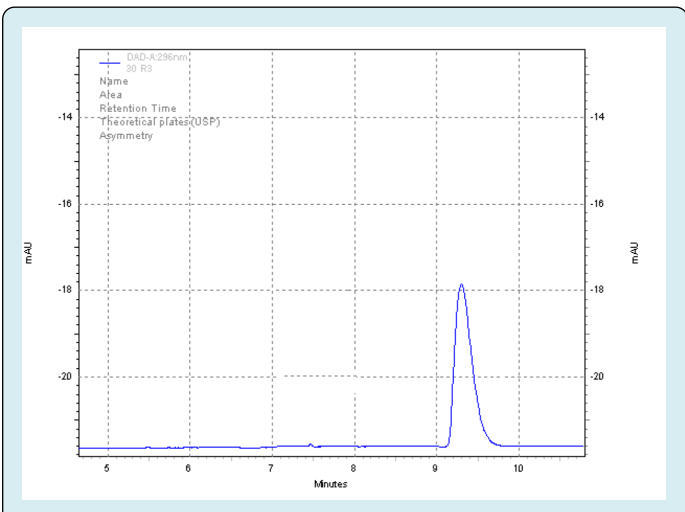

Figure 4: Chromatogram of Flecainide Acetate Showing Retention Time 9.3min.

Validation of Proposed Methods A, B and C

The proposed UV and HPLC methods A, B and C were validated across various parameters like linearity, accuracy, precision, ruggedness, sensitivity, repeatability, bulk and pharmaceutical formulation assay according to ICH guidelines [11].

Linearity

The concentration range 20-120µg/ml for method A and method B, while for method C the linearity concentration range 10-50µg/ml.

Accuracy

To the pre-analyzed sample solutions, a known amount of stock standard solution was added at different levels, i.e. 80%, 100%, and 120%. The solutions were re-analyzed by the proposed methods.

Precision

The precision of the methods was studied as intra-day and inter-day variations. In Method A and B precision was determined by analyzing the 40, 60, and 80µg/ml of FA solutions as intra-day and inter-day variations. While in method C precision was determined by analyzing 20, 30, and 40µg/ml of FA solutions as intra-day and inter-day variations.

Ruggedness

The ruggedness of the proposed methods was determined for 60µg/ml concentrations of drug in Method A and B and 30µg/ml in Method C by analysis of aliquots from a homogenous slot by two analysts using the same operational and environmental conditions.

Sensitivity

The sensitivity of measurements of FA by the use of the proposed methods was estimated in terms of the limit of quantification (LOQ) and the limit of detection (LOD). The LOQ and LOD were calculated using equation $LOD=3.3\times N/B$ and $LOQ=10\times N/B$, where 'N' is the standard deviation of the AUC of the drugs (n=3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve.

Repeatability

Repeatability was determined by analyzing 60µg/ml concentration of FA solution for six times for methods A and B and 30µg/ml for method C.

Determination of Flecainide Acetate in bulk

A quantity of powder equivalent to 60µg/ml was transferred into a 100ml volumetric flask containing 30 ml of water, sonicated for 15min, the volume was adjusted to the mark using the same solvent and filtered through Whatman filter paper no. 41. An appropriate volume 6 ml was transferred into a 10ml volumetric flask and volume was adjusted to the mark to obtain the desired concentration of 10µg/ml. The AUC was recorded at selected wavelengths for Method A while in Method B, AUC of the first-order derivative spectrum were recorded in between selected wavelength ranges and for method C concentration of 30µg/ml was injected in system and the concentration of the drug was determined from the respective linear regression equations.

Application of Proposed Method for Pharmaceutical Tablet Formulation

The pharmaceutical tablet formulation, Flecarite (Torrent) 20 tablets were accurately weighed, average weight determined and ground into fine powdered. A quantity of powder equivalent to 60µg/ml was transferred into a 100ml volumetric flask containing 30ml of water, sonicated for 15min, volume was adjusted to the mark using the same solvent and filtered through Whatman filter paper no. 41. An appropriate volume 6ml was transferred into a 10ml volumetric flask and the volume were adjusted to the mark to obtain the desired concentration of 10µg/ml. The AUC was recorded at selected wavelengths for method A while in method B, AUC of the first-order derivative spectrum was recorded in between selected wavelength ranges and 30µg/ml injected in HPLC system (Method C).The concentration of the drug was determined from the respective linear regression equations.

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Results and Discussion

The proposed methods (A, B and C) was validated across the various parameters like linearity, accuracy, precision, ruggedness, sensitivity, repeatability, bulk and pharmaceutical formulation assay as per ICH guidelines.

Linearity

The linear regression data for the calibration curves showed a good linear relationship over the concentration range 20-120µg/ml for Method A and Method B. The linear regression equation was found to be y= 0.0463x–0.0403 and y = 0.0373x+0.1652 for AUC zero order and AUC first order respectively while a good correlation coefficient (r^2=0.9995 and r^2=0.9993) for both the methods A and B (Figures 5a & 5b) and for method C the linearity concentration range 10-50µg/ml and the linear regression equation was found to be y= 327.29x+5649.7 with r^2=0.9992 (Figure 6).

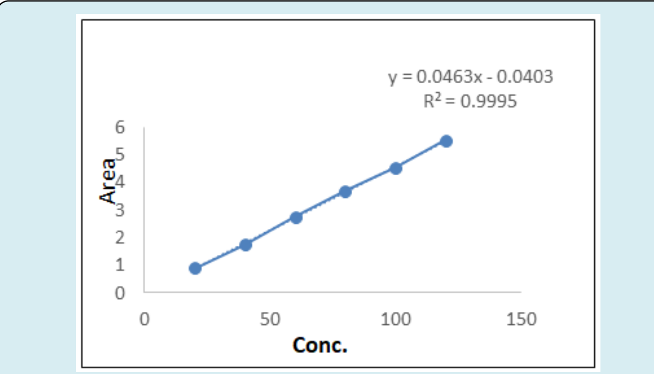

Figure 5a: Linearity Curve of Flecainide Acetate by AUC Zero Order Derivatives.

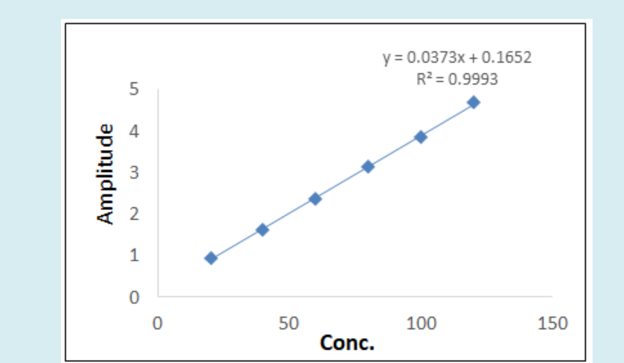

Figure 5b: Linearity Curve of Flecainide Acetate by AUC First Order Derivatives.

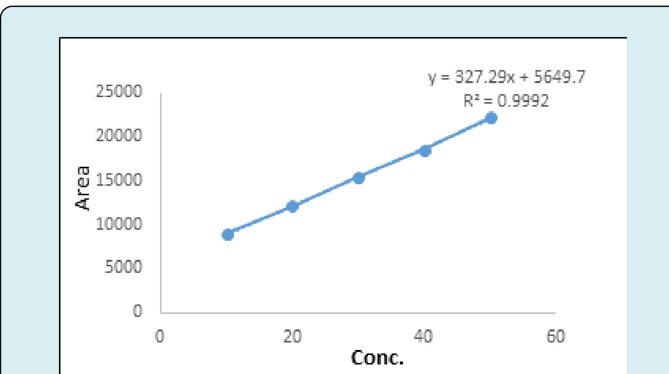

Figure 6: Linearity Curve of Flecainide Acetate by RP-HPLC.

The results of linearity are expressed in Table 1.

Method A		Method B		Method C	
Conc.(µg/ml)	Area	Conc.(µg/ml)	Amplitude	Conc.(µg/ml)	Area
20	0.9103	20	0.9503	10	9015
40	1.7547	40	1.6393	20	12155
60	2.7497	60	2.3803	30	15445
80	3.7031	80	3.1376	40	18540
100	4.5489	100	3.8512	50	22187
120	5.5188	120	4.6912		

Table 1: Linearity Studies of Flecainide Acetate.

Accuracy

The solutions were reanalyzed by proposed method. The mean % recovery was found to be in the range of 98.45 to 99.99% for all three methods with % RSD less than 2 indicate that the methods were precise. The results are expressed in Table 2.

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Methods	at 80%		at 100%		at 120%	
	% recovery ±SD	%RSD	% recovery ±SD	%RSD	% recovery ±SD	%RSD
A	98.45± 0.71	0.72	99.40± 1.42	1.42	99.99± 0.44	0.44
B	98.88± 0.38	0.38	99.70± 0.80	0.8	99.68± 1.09	1.09
C	98.62± 0.64	0.64	99.40± 1.03	1.04	99.29± 0.67	0.68

*(n=3)

Table 2: Accuracy Studies of Flecainide Acetate.

Precision

The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). These results show reproducibility of the assay. The % RSD values found to be less than 2 indicate that the methods were precise for the determination of drugs in formulation. The results are expressed in Table 3.

Methods	Conc. (µg/ml)	Intra-day precision		Inter-day precision	
		% Amount found*± SD	% RSD	% Amount found*± SD	% RSD
A	40	98.35± 0.65	0.66	98.48± 0.47	0.48
	60	99.36± 0.76	0.77	99.31± 0.72	0.73
	80	99.12± 0.13	0.13	99.05± 0.11	0.11
B	40	98.25± 0.55	0.56	98.31± 0.50	0.51
	60	98.44± 0.59	0.6	98.56± 0.61	0.62
	80	99.40± 0.62	0.62	99.50± 0.67	0.68
C	20	98.66± 0.86	0.87	98.71± 0.83	0.84
	30	98.82± 0.82	0.83	98.44± 1.13	1.14
	40	98.36± 0.14	0.14	98.68± 0.33	0.34

*(n=3)

Table 3: Precision Studies of Flecainide Acetate.

Ruggedness

Ruggedness of the proposed methods was determined for selected concentrations. The results were in the acceptable range for all the three methods and % RSD was found to be less than 2, indicating that the method is rugged. The detail results are tabulated as in Table 4.

Methods	Conc. (µg/ml)	Analyst I		Analyst II	
		% Amount found*± SD	% RSD	% Amount found*± SD	% RSD
A	60	99.07± 1.04	1.05	98.56± 0.84	0.85
B	60	98.54± 0.70	0.71	99.04± 1.05	1.06
C	30	98.86± 0.81	0.82	98.47± 0.83	0.84

*(n=6)

Table 4: Ruggedness Studies of Flecainide Acetate.

Repeatability

Repeatability was determined for selected concentrations of FA solution for six times and the % amount determined with % RSD less than 2 for all the three methods. The results are expressed in Table 5.

Methods	Amount taken (µg/ml)	Amount found*	% Amount found*± SD	% RSD
A	60	59.07	98.45± 0.87	0.88
B	60	59.14	98.56± 0.66	0.67
C	30	29.63	98.77± 0.84	0.85

Table 5: Repeatability Studies of Flecainide Acetate.

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Analysis of Flecainide Acetate in Bulk

The concentrations of the drug were calculated from linear regression equations. The % amount found was within 98.66% to 99.15% with % RSD less than 2 for all the three methods. The results are expressed in Table 6.

Methods	Amount taken (µg/ml)	Amount found*	% Amount found*± SD	% RSD
A	60	59.42	99.03± 1.03	1.04
B	60	59.49	99.15± 0.90	0.91
C	30	29.59	98.66± 0.99	1

*(n=6)

Table 6: Analysis of Flecainide Acetate in Bulk.

Analysis of Flecainide Acetate in Tablet Formulation

The spectrum was recorded at 296nm. The concentrations of the drug were calculated from linear regression equation. The % amount was found around 98% with % RSD less than 2 for all the three methods. The results are expressed in Table 7.

Methods	Amount taken (µg/ml)	Amount found*	% Amount found*± SD	% RSD
A	60	59.11	98.52± 0.80	0.81
B	60	59.2	98.67± 0.79	0.8
C	30	29.59	98.64± 0.68	0.69

*(n=6)

Table 7: Analysis of Flecainide Acetate in Tablet Formulation.

Sensitivity

The LOD and LOQ for selected drug were found to be 0.20 and 0.61µg, respectively, for method A, and 0.32 and 0.97µg, respectively for method B while it is found to be 0.03 and 0.12 for method C. The results are expressed in Table 8.

Methods	Linear Regression Equation	LOD (µg/ml)	LOQ (µg/ml)
A	$y = 0.0463x - 0.0403$ ($r^2 = 0.9995$)	0.2	0.61
B	$y = 0.0373x + 0.1652$ ($r^2 = 0.9993$)	0.32	0.97
C	$y = 327.29x + 5649.7$ ($r^2 = 0.9992$)	0.03	0.12

Table 8: Sensitivity Studies of Flecainide Acetate.

Conclusion

The spectrophotometric and chromatographic methods are developed and validated successfully for estimation of Flecainide acetate. The determination of drug candidate was done by taking bulk as well as in pharmaceutical tablet formulation. The results of the analysis of pharmaceutical formulation by the proposed methods are reproducible and reliable. This indicates that there is no interference of excipients. All these developed spectrophotometric and chromatographic methods are found to be simple, accurate, precise, and economical when validated as per ICH guidelines. Thus the proposed method can be used for routine analysis of Flecainide acetate.

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Role of Citrus Pectin in Biological Activity: A Review

D.S. Kachare*[1], P. K. Ghadge[2], Sachin S. Mali[3]

Researcher Student[1], Assistant Professor[2], Principal[3]

Department of Pharmacology[1, 2], Department of Pharmaceutics[3]

Yashoda Technical Campus, Wadhe, Satara, India[1, 2],

Y. D. Mane Institute of Pharmacy (Diploma), Kagal, Kolhapur, India[3]

Corresponding Author's email id: *dhanshrikachare44@gmail.com[*1]*

Abstract

Pectin is a naturally occurring biopolymer. It has been used successfully for many years in the food and beverage industry as a thickening agent, a gelling agent and a colloidal stabilizer. In this review, study of the role of pectin polysaccharides, including its various pharmacological activity, such as its immuno-regulatory, anti-inflammatory, hypoglycemic, antibacterial, antioxidant and antitumor activities, have been summarized. The review provides natural sources, chemical structures, biological activities, and practical applications in the food industry as well as pharmacology and different branches of medicine. Pectin has become an essential part of the research and development of natural herbs and health products due to their wide availability.

***Keywords**: - Citrus Pectin, Source, extraction, Biological activity, glycogen regulation.*

INTRODUCTION

French chemist Louis Nicolas Vauquelin in 1790 in tamarind fruit, discovered pectin in citrus fruits (Vauquelin, 1790). Henri Braconnot introduced the term "pectin" because of the gelling properties of these substances (Braconnot, 1825). Pectin is usually obtained from the residues of plant material after extracting the juice (apple or citrus peel) or sugar (sugar beet). This review will first describe the source and production, chemical structure and general properties of pectin. Pectin is the methylated ester of polygalacturonic acid, contains 1, 4-linked α-D-galacturonic acid residues.

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STRUCTURE OF PECTIN

Pectin is a polysaccharide with a core consisting of α-1,4-linked D-galacturonic acid and α-1, 2-L-rhamnose, large number of neutral sugars, including arabinose, galactose, and lesser amounts of other sugars. The structural classification of pectin includes: homogalacturonan (HG), rhamnogalacturonan I (RG-I), and substituted galacturonans such as rhamnogalacturonan II (RG-II) (Figure 1). It composed of as many as 17 different monosaccharides and more than 20 different linkages. (Minzanova, Salima T et al. 2018)

GENERAL PROPERTIES OF PECTIN

Pectins are soluble in warm water. Monovalent cation salts of pectinic and pectic acids are usually soluble in water; di- and trivalent cations salts are weakly soluble or insoluble. Dry powdered pectin, when added to water, has a tendency to hydrate very rapidly, forming clumps.

Most important use of pectin is based on its ability to form gels. HM-pectin forms gels with sugar and acid. HM-pectin, unlike LM-pectin, does not contain sufficient acid groups to gel or precipitate with calcium ions, although other ions such as aluminium or copper cause precipitation under certain conditions. (Rolin C 1993), (Hercules Incorporated 1999). (Raj et al. 2012)

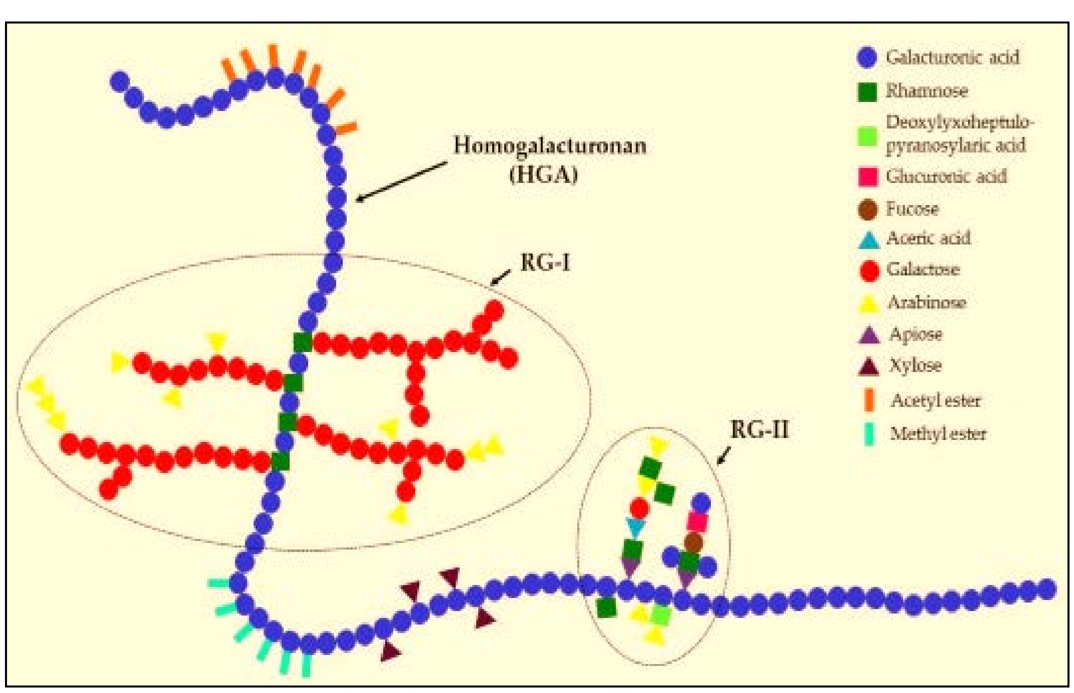

***Fig.1**. A scheme of primary structure of pectin*

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METHODSOF PECTIN PREPRATION

Sample Preparation

The samples (Cola milleni, Irvingia gabonensis and Theobroma cacao) peel and husk were sun dried for one week and making powder using blender. The powdered samples were sieved with a fine mesh of size 14mm. The sieved samples were kept in an air tight container prior to extraction process.

Extraction of Pectin (Oloye, 2013)

Extraction of pectin from the samples was performed under acid condition. The dried powder pectin was extracted by mixing with acidified distilled water inside a water bath at different temperatures ranges from 50 - 100°C and different time from 30 – 150 mins. After contact time reflux, the samples were filtered through cheesecloth and cooled; it was then centrifuged for 20 mins at 3,500 rpm. Ethanol 96% was added to the supernatant and allowed to stand for one hour for pectin precipitation. The precipitated pectin was separated by filtration, washed thrice its volumes with absolute ethanol and washed twice with water to remove impurity. The extracts were separately dried in an oven at 50°C and the pectin yield was determined. The dried pectin samples were stored in aluminium foils at 4°C until used.

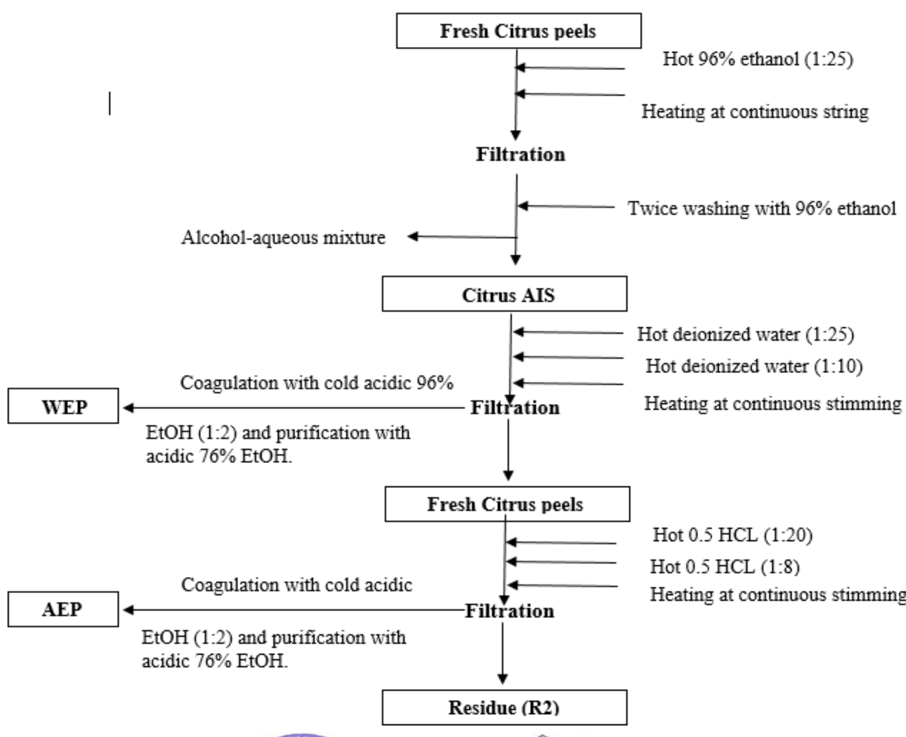

Fig.2. Preparation of pectin extraction from orange and lemon peels

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Chemical Constituents: The neutral sugars that essentially form the side chains on the pectin molecules are namely: galactose, arabinose, xylose, and fructose. They also contain Saponin, Tannin, Alkaloid, Flavonoid, Steroid, Terpenoid, Glycoside and Phenol. (Oloye, 2013)

ROLE OF CITRUS PECTIN IN BIOLOGICAL ACTIVITY

Immunomodulatory Activity:

Immunomodulators are natural or synthetic substances that have a regulating effect on the immune system. Plant-derived polysaccharides, including pectin can directly activate the immune function of macrophages, promote the production of cytokines, and therefore regulate the immune system on multiple levels. The immunomodulatory activities of the oligomer fractions are still observed in the studies. Enzymatic digestion of pectin, which also show the value of the back bone of pectin.

The carbohydrate chain of pectin determines immunosuppressive activity. It was found that pectin containing more than 80% of galacturonic acid residues suppress the activity of macrophages and inhibit the delayed-type hypersensitivity reaction. In addition, the branched region of the pectin macromolecule mediates the stimulation of phagocytosis and increased production of antibodies. (Popov & Ovodov, 2013)

Anti-inflammatory Effect:

Inflammation has been considered as a main risk factor for different progressive illnesses in human beings, such as neurological disorders, cancer, metabolic diseases, and cardiovascular disease, and a primary strategy to prevent these diseases is to target the reduction of chronic inflammation. (Leivas C.L et al., 2016)The intake of dietary fibers such as plant cell wall polysaccharides enhances the efficiency of treatment of inflammatory processes. Much attention has been focused in recent years on pectin.

Popov et al. has studied the anti-inflammatory activity of citrus pectin in vivo after oral administration in mice. (Popov S.V et al., 2013)Three models of inflammation were used: cytokine production by blood leukocytes in response to lipopolysaccharide, acetic acid-induced colitis, and endotoxin shock.

The results of the study demonstrate that low methyl-esterified citrus pectin inhibits local and systemic inflammation, while

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pectin with a higher degree of esterification can inhibit intestinal inflammation.

Antioxidant Activity:

Oxidation is vital to plenty of organisms that can generate energy to supply biological processes. In normal circumstances, free radicals govern cell growth, and suppress viruses and bacteria. Nevertheless, in large quantities and without regulation, the production of free radicals induced by oxygen cause cell damage, which renders the pathological progressions. The oxidative stress is associated with chronic obstructive pulmonary disease, asthma, diabetes, inflammation, cardiovascular diseases, and myocardial infarction. (Wang, J et al., 2016)

Antitumor Activity:

Many in vitro and in vivo studies concerning the antitumor activity of native and modified pectin revealed a decrease of adhesion and cell proliferation, as well as the induction of apoptosis and migration. (Bush, P et al., 2014) Maxwell et al. have assessed pectin from different sources (potato, sugar beet, larch, and citrus) for effects against colon cancer cells. (Maxwell,E.G et al.,2015) Extracts of potato pectin lowered the proliferation of colon cancer cells by the alteration of dose.

Sugar beet pectin extracts presenting various structures of pectin showed high anti-proliferative action against colon cancer cells. (Maxwell, E.G et al., 2016) The alkali treatment of pectin surged the antitumor activity of sugar beet pectin due to an apoptosis promotion. The pectic polysaccharide from apple can induce the death of cancer cells death and suppress the growth of tumors in vivo, as Delphi and Sepehri described. (Delphi, L et al., 2016)

Hypoglycemic / Anti-diabetic Effect:

Research was conducted to study the anti-diabetic effect of citrus pectin in diabetic rats and the potential benefits of citrus pectin to produce anti-diabetic effects in cases of T2DM caused by a low-dose streptozotocin and a fat-laden diet. Many tests have demonstrated that anti-diabetic polysaccharides effectively improve glucose tolerance.

Low-dose citrus pectin (500 mg/kg bw per day), the medium-dose citrus pectin (1000 mg/kg bw per day) and the high-dose

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citrus pectin (2000 mg/kg bw per day) intragastrically administered.

Citrus pectin reduced fasting blood glucose levels, benefited hyperlipidemia, and refined hepatic glycogen content glucose tolerance in the diabetic rats. Citrus pectin modulated the expression of the basic proteins in the PI3K/Akt signaling pathway, which could have influenced the enhancement of insulin sensitivity in the diabetic rats, possibly by signifying the anti-diabetic effect of citrus pectin. (Liu, Dong, Yang, & Pan, 2016)

Neuroprotective Effect:

We investigated the neuroprotective activities of ginseng pectin (GP) against hydrogen peroxide (H_2O_2)-induced neuronal toxicity in different neuronal cells. GP protects neuronal cells from hydrogen peroxide-induced cell death. GP protects cortical neuron cell neurites from degeneration. GP neuroprotective effect occurs through the activation of ERK/MAPK and Akt survival signaling pathways. Ginsenoside Rb1 has been shown to protect neuronal cells against hydrogen peroxide-induced cell damage possibly by scavenging free radicals, inhibiting the production of nitric oxide, preventing lipid peroxidation and avoiding decrease in SOD activity.

GP showed similar protective effects on neuronal cells against H_2O_2-induced oxidative stress via regulating the pro-survival ERK/MAPK and Akt pathways. Moreover, GP preserved the structural integrity of neurons, suggesting that it may be a new neurotrophin. In conclusion, GP appears to be anti-oxidant without side-effects, which may eventually lead to further development of therapeutics for neurodegenerative diseases. (Fan et al., 2012)

Glycogen regulation

Pectin decreased PKC (protein kinase-c) activity in liver and increased PKC activity in brain. (Kramer HK, 1997) Pectin also enhanced glycogenesis and reduced glycogenolysis. Activation of PKC stimulate the serotonin receptor or transporter in brain. (Vijayalakshmi, 2014).MCP prevents blood-brain barrier disruption possibly by inhibiting galectin-3.

DISCUSSION

Pectin structure provide significant immunomodulatory properties. However, future research studies necessary to verify

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the immune activity in vivo and determine howthemechanismsofpectinaffectmacrophagesandotherimmunocytesforsafeclinical applications.

Studies of the antimicrobial properties of pectins show a general tendency for the development of nanocomposites and nanoemulsions on their base. Both exhibit noticeable inhibitory effects, mainly against Gram-negative Escherichia coli and Gram-positive Staphylococcus aureus. The analysis of the reviewed studies shows that pectin polysaccharides from various source demonstrate antioxidant activity. It was also shown that pectins of diverse chemical structure (HG, RG-I, RG-II) exhibit antioxidant properties. Hypoglycemic activity of pectins is useful for the development of low-toxicity antidiabeticagents.

Drugs based on dietary and medicinal plants don't cause side effects, which helps pectin promising for further research. Anti-inflammatory properties of pectin is given great attention in the literature, because pectin have a great potential for anti-inflammatory multi-purpose therapy. Another fast-growing field of pectin useful application is anti-cancer therapy, which is due to safety of pectin and its derivatives. However, the lack of research on pectin polysaccharide protection mechanisms and clinical trials has limited the application of pectin in the field of medicine thus far.

CONCLUSION

Recently updated knowledge about Citrus pectinplay beneficial role in various biological activity. Pectin extracted from Citrus fruits, Cola milleni, Irvingia gabonensis and Theobroma cacao. Pectin also enhanced glycogenesis and reduced glycogenolysis .Results of a study of the bioactivity of pectin polysaccharides, including its various pharmacological action, such as its immunoregulatory, anti-inflammatory, hypoglycemic, antibacterial, antioxidant and antitumor activities, Neuroprotective have been summarized.

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Sodium alginate cross-linked polymeric microbeads for oral sustained drug delivery in hypertension: Formulation and evaluation

Kalbhare Shankar B.[1,*], Bhandwalkar Mandar J.[1], Pawar Rohit K.[1], Sagre Abhirup R.[2]

[1]Department of Pharmaceutics, YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Wadhe Satara-415001 Dist-Satara (M.S.), India

[2]Department of Pharmacology, YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Wadhe Satara-415001 Dist-Satara (M.S.), India

*Corresponding Author E-mail: kirankal786@gmail.com

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Abstract

The Oral controlled drug delivery system represent the most popular form of sustain drug delivery system for the obvious advantages of oral route of drug administration. Such systems release the drug with constant or variable release rates. The oral controlled release system show a typical pattern of drug release in which the drug concentration is maintain in the therapeutic windows for prolonged period of time (sustain release), there by insuring sustained therapeutic action. They are used as single dosage form. λMax of losartan potassium was found to be at 205.3 nm. All formulation of Losartan potassium micro beads showed the particle size between 0.61 to 0.77mm. The lowest % entrapment efficiency was obtained in formulation F1 and highest was found to be formulation F3. The result demonstrated that effect on % entrapment efficiency with in decreasing concentration of sodium alginate. The drug releases of F 1, F 2 and F 3 formulations is found to be 50.2% to 98.52%, 49.21% to 98.23% and 43.21% to 98.52% respectively. Among all formulations, F 3 was show maximum drug release i.e. 98.51% after 12 hr. It was observed that extent of drug release is attributed to the increase in amount of sodium alginate. All the formulation of microbeads produced the optimum swelling [...] 156–267. The minimum swelling ratio of formulation F1 and higher swelling ratio of formulation F3 is obser[...] involves preparation and evaluation of sustained release of microbeads of losartan potassium employing sodium alginate as natural polymer. The technique employed for

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Analytical Method Development and Validation of Flecainide Acetate by Chromatographic and Spectrophotometric Techniques

Redasani VK[1]*, Agrawal YO[2], Jagtap MS[2], Mahajan HS[2] and Surana SJ[2]

[1]Faculty of Pharmacy, YSPMs Yashoda Technical Campus, India

[2]R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur, India

***Corresponding author:** Vivekkumar K Redasani, Faculty of Pharmacy, YSPMs Yashoda Technical Campus, Satara [India], Tel: +918421456827; Email: vivek.redasani@gmail.com

Research Article

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Abstract

The aim of the present work is to establish three simple, economical and rapid methods for the quantification of Flecainide acetate in bulk drug and in tablets formulation. This study is designed to validate and developed spectroscopic and chromatographic methods as per ICH guidelines. In Method A, area under curve (AUC) of zero-order spectrum was recorded between 277.00 and 310.00nm. Linearity was found in the concentration range of 20-120µg/ml. In Method B, zero-order spectra were derivatized into first-order and the AUC was recorded between 280.00 and 310.20 nm. The linearity was found in the conc. range of 20-120µg/ml. In Method C, separation was achieved using RP-HPLC by gradient elution using a C18 Qualisil BDS (250mm×4.5mm×5µm) column, a mobile phase consisting of water: acetonitrile (60:40 v/v) with mobile phase pH adjusted with orthophosphoric acid (pH 3.0), a flow rate of 1.0ml/min and UV detection at 296nm. The linearity was obtained in the concentration range of 10-50µg/ml. The developed methods are validated successfully across various parameters in accordance with ICH guidelines. Thus, the proposed methods can be used for routine analysis of Flecainide acetate as it does not showed any interference of excipients when estimated in pharmaceutical formulations.

Keywords: Flecainide Acetate; Spectrophotometric; Chromatographic; Area Under Curve; Derivative-Spectrophotometry; Validation; ICH Guidelines

Abbreviations: AUC: Area under Curve; FA: Flecainide Acetate; LOQ: Limit of Quantification; LOD: Limit of Detection.

Introduction

Flecainide Acetate (FA) is chemically designated as N-(2-piperidinylmethyl)-2, 5-bis (2, 2, 2–trifluoroethoxy) benzamide acetate having molecular formula: $C_{19}H_{24}F_6N_2O_5$ with molecular weight: 474.4g/mol (Figure 1) [1]. It is a white solid powder with melting point 146-152°C [2]. It is an antiarrhythmic agent (class IC) causing a decreased in intra-cardiac conduction velocity in all parts of the heart [3]. It is used for the treatment of tachyarrhythmia, atrial fibrillation, supraventricular tachycardia and ventricular tachycardia by blocking Na+ current delayed rectifier K+ current [classified as a Na+ channel blocker drug] [4]. Flecainide also has local anesthetic effects. It selectively increases anterograde and retrograde accessory pathway refractoriness. The action of Flecainide in the heart prolongs the PR interval and widens the QRS complex. The effect on the JT interval is insignificant as Flecainide does not lengthen ventricular repolarization [5]. The drug is official in the European [6], British [7] and US pharmacopeias [8]. Several chromatographic methods have been reported in the literature for the determination of FA in its bulk powder, in pharmaceutical formulations or in the presence of its enantiomers, metabolites or other

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antiarrhythmic drugs [4].

Figure 1: Chemical Structure of Flecainide Acetate.

Analysis part is an important for formulation development of any drug molecule. A suitable and validated method should be vacant for the drug delivery system for analysis of bulk drug and formulation [9]. The developed methods were validated for accuracy, precision, ruggedness, and sensitivity. Accordingly, the objective of this study was to develop and validate the simple spectrophotometric method for the estimation of FA in bulk and tablets as per ICH guidelines.

Experimental

Instrumentation

The Shimadzu double beam UV-VIS spectrophotometer with spectra manager software UV Probe 2.21 with 10mm quartz cells was used. The chromatographic separation was achieved by using Agilent system consisting photodiode array detector, C_{18} Qualisil BDS (250mm×4.5mm×5µm) column [10]. All weights were taken on an electronic balance (Model Shimadzu AUX 120).

Chemicals

The gift sample of FA was obtained from Indeus Life Sciences Pvt. Ltd, Mumbai. Analytical grade solvents and reagents were purchased from Merck specialties Pvt. Ltd. Mumbai (India). Double distilled water filtered through the membrane filter was used. Flecarite tablets each containing 100mg of active drug were purchased from the local market.

Preparation of Standard Stock Solution

The standard stock solution was prepared by dissolving 10mg of FA in the 100ml of water, sonicated and to obtain the concentration of 100µg/ml.

Method A: Area under Curve Zero Order UV Spectrophotometric Method: Concentration of 10µg/ml was prepared by diluting 1ml of standard stock solution with 10ml of water. This solution was scanned in the UV-visible range 400-200nm. FA showed the maximum absorbance at 296 nm as shown in and the AUC of the zero order spectrums was recorded between the 277.00-310.00nm (Figure 2).

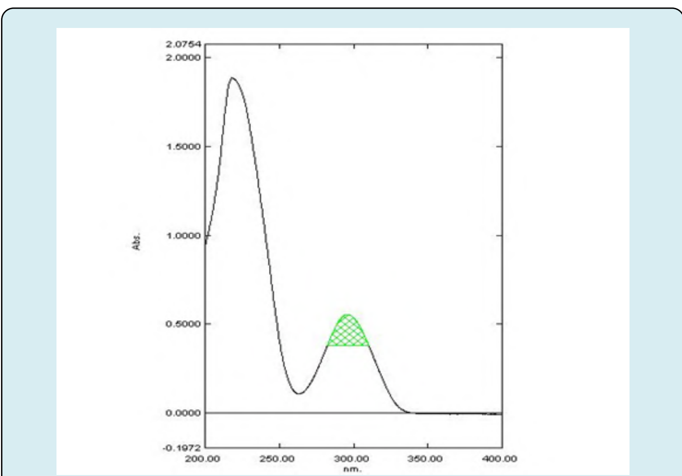

Figure 2: AUC Zero Order Derivative Spectrum of Flecainide Acetate in Water.

Method B: Area Under Curve First Order UV Spectrophotometric Method: The zero order spectrums were derivatized into first order spectrum and the AUC was recorded between 280.00-310.20nm as shown in Figure 3.

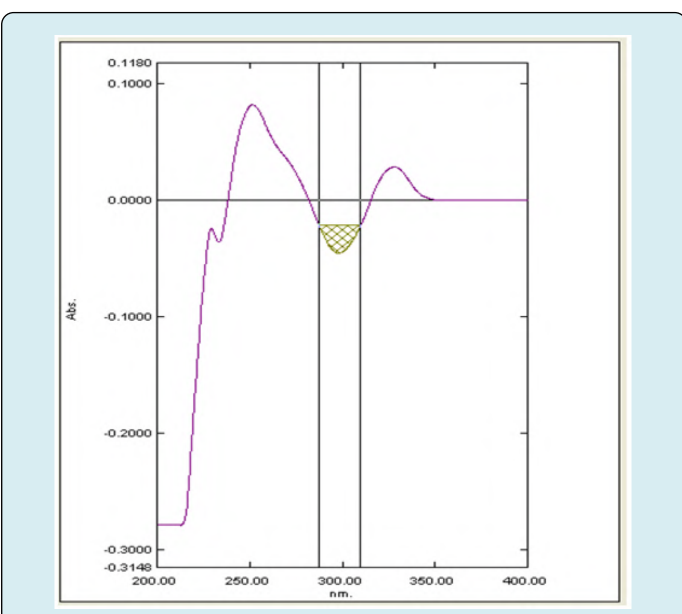

Figure 3: AUC First Order Derivative Spectrum of Flecainide Acetate in Water.

Method C: RP-HPLC Method: In Method C RP-HPLC method in which separation was achieved by gradient elution using a C_{18} Qualisil BDS (250mm×4.5mm×5µm) column, a mobile phase consisting of water: acetonitrile (60:40 v/v) with mobile phase pH adjusted with orthophosphoric acid (pH 3.0), a flow rate of 1.0ml/min and UV detection at 296nm. The linearity was obtained in the conc. range of 10-50µg/ml. The retention time of Flecainide acetate was 9.3min (Figure 4).

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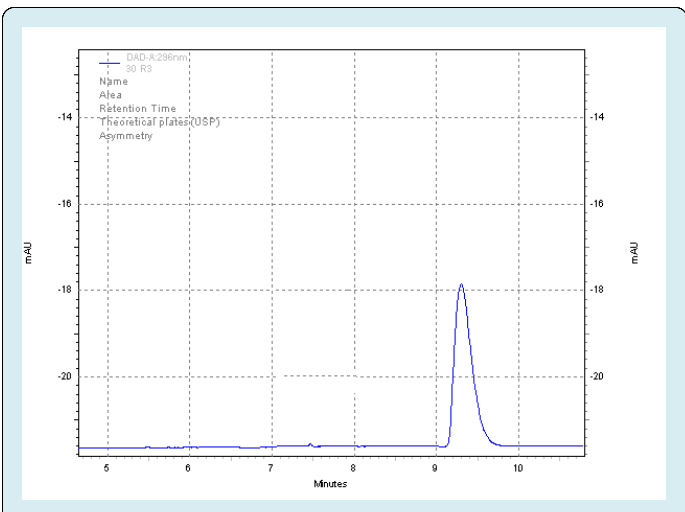

Figure 4: Chromatogram of Flecainide Acetate Showing Retention Time 9.3min.

Validation of Proposed Methods A, B and C

The proposed UV and HPLC methods A, B and C were validated across various parameters like linearity, accuracy, precision, ruggedness, sensitivity, repeatability, bulk and pharmaceutical formulation assay according to ICH guidelines [11].

Linearity

The concentration range 20-120µg/ml for method A and method B, while for method C the linearity concentration range 10-50µg/ml.

Accuracy

To the pre-analyzed sample solutions, a known amount of stock standard solution was added at different levels, i.e. 80%, 100%, and 120%. The solutions were re-analyzed by the proposed methods.

Precision

The precision of the methods was studied as intra-day and inter-day variations. In Method A and B precision was determined by analyzing the 40, 60, and 80µg/ml of FA solutions as intra-day and inter-day variations. While in method C precision was determined by analyzing 20, 30, and 40µg/ml of FA solutions as intra-day and inter-day variations.

Ruggedness

The ruggedness of the proposed methods was determined for 60µg/ml concentrations of drug in Method A and B and 30µg/ml in Method C by analysis of aliquots from a homogenous slot by two analysts using the same operational and environmental conditions.

Sensitivity

The sensitivity of measurements of FA by the use of the proposed methods was estimated in terms of the limit of quantification (LOQ) and the limit of detection (LOD). The LOQ and LOD were calculated using equation LOD=3.3×N/B and LOQ=10×N/B, where 'N' is the standard deviation of the AUC of the drugs (n=3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve.

Repeatability

Repeatability was determined by analyzing 60µg/ml concentration of FA solution for six times for methods A and B and 30µg/ml for method C.

Determination of Flecainide Acetate in bulk

A quantity of powder equivalent to 60µg/ml was transferred into a 100ml volumetric flask containing 30 ml of water, sonicated for 15min, the volume was adjusted to the mark using the same solvent and filtered through Whatman filter paper no. 41. An appropriate volume 6 ml was transferred into a 10ml volumetric flask and volume was adjusted to the mark to obtain the desired concentration of 10µg/ml. The AUC was recorded at selected wavelengths for Method A while in Method B, AUC of the first-order derivative spectrum were recorded in between selected wavelength ranges and for method C concentration of 30µg/ml was injected in system and the concentration of the drug was determined from the respective linear regression equations.

Application of Proposed Method for Pharmaceutical Tablet Formulation

The pharmaceutical tablet formulation, Flecarite (Torrent) 20 tablets were accurately weighed, average weight determined and ground into fine powdered. A quantity of powder equivalent to 60µg/ml was transferred into a 100ml volumetric flask containing 30ml of water, sonicated for 15min, volume was adjusted to the mark using the same solvent and filtered through Whatman filter paper no. 41. An appropriate volume 6ml was transferred into a 10ml volumetric flask and the volume were adjusted to the mark to obtain the desired concentration of 10µg/ml. The AUC was recorded at selected wavelengths for method A while in method B, AUC of the first-order derivative spectrum was recorded in between selected wavelength ranges and 30µg/ml injected in HPLC system (Method C).The concentration of the drug was determined from the respective linear regression equations.

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Results and Discussion

The proposed methods (A, B and C) was validated across the various parameters like linearity, accuracy, precision, ruggedness, sensitivity, repeatability, bulk and pharmaceutical formulation assay as per ICH guidelines.

Linearity

The linear regression data for the calibration curves showed a good linear relationship over the concentration range 20-120μg/ml for Method A and Method B. The linear regression equation was found to be y= 0.0463x–0.0403 and y = 0.0373x+0.1652 for AUC zero order and AUC first order respectively while a good correlation coefficient (r^2=0.9995 and r^2=0.9993) for both the methods A and B (Figures 5a & 5b) and for method C the linearity concentration range 10-50μg/ml and the linear regression equation was found to be y= 327.29x+5649.7 with r^2=0.9992 (Figure 6).

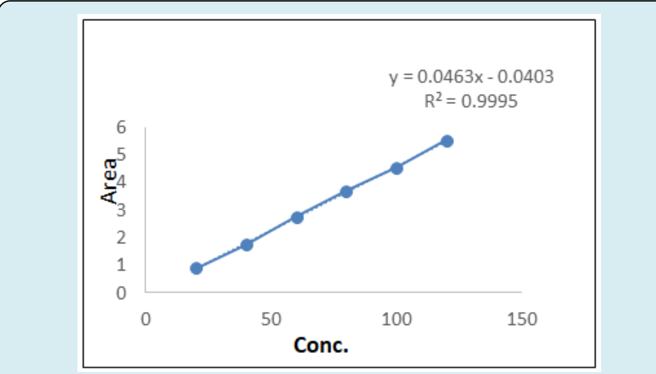

Figure 5a: Linearity Curve of Flecainide Acetate by AUC Zero Order Derivatives.

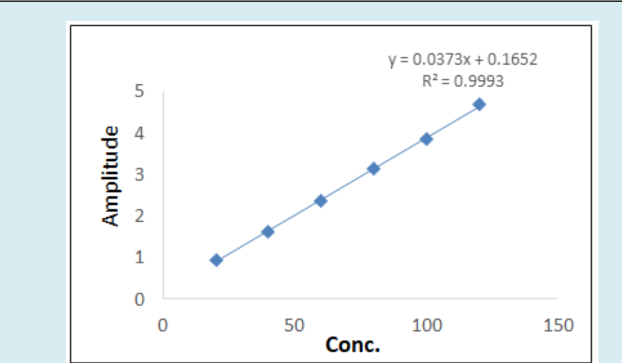

Figure 5b: Linearity Curve of Flecainide Acetate by AUC First Order Derivatives.

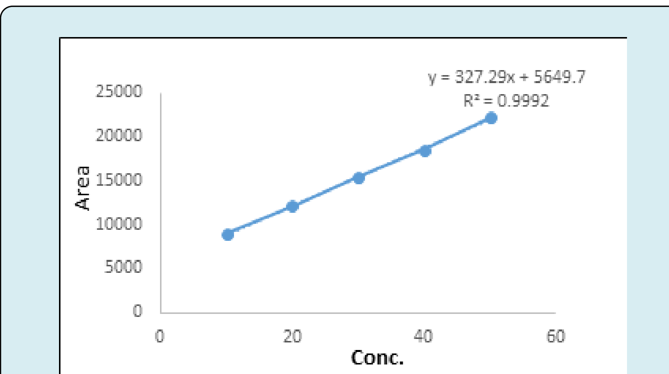

Figure 6: Linearity Curve of Flecainide Acetate by RP-HPLC.

The results of linearity are expressed in Table 1.

Method A		Method B		Method C	
Conc.(μg/ml)	Area	Conc.(μg/ml)	Amplitude	Conc.(μg/ml)	Area
20	0.9103	20	0.9503	10	9015
40	1.7547	40	1.6393	20	12155
60	2.7497	60	2.3803	30	15445
80	3.7031	80	3.1376	40	18540
100	4.5489	100	3.8512	50	22187
120	5.5188	120	4.6912		

Table 1: Linearity Studies of Flecainide Acetate.

Accuracy

The solutions were reanalyzed by proposed method. The mean % recovery was found to be in the range of 98.45 to 99.99% for all three methods with % RSD less than 2 indicate that the methods were precise. The results are expressed in Table 2.

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Methods	at 80%		at 100%		at 120%	
	% recovery ±SD	%RSD	% recovery ±SD	%RSD	% recovery ±SD	%RSD
A	98.45± 0.71	0.72	99.40± 1.42	1.42	99.99± 0.44	0.44
B	98.88± 0.38	0.38	99.70± 0.80	0.8	99.68± 1.09	1.09
C	98.62± 0.64	0.64	99.40± 1.03	1.04	99.29± 0.67	0.68

*(n=3)

Table 2: Accuracy Studies of Flecainide Acetate.

Precision

The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). These results show reproducibility of the assay. The % RSD values found to be less than 2 indicate that the methods were precise for the determination of drugs in formulation. The results are expressed in Table 3.

Methods	Conc. (µg/ml)	Intra-day precision		Inter-day precision	
		% Amount found*± SD	% RSD	% Amount found*± SD	% RSD
A	40	98.35± 0.65	0.66	98.48± 0.47	0.48
	60	99.36± 0.76	0.77	99.31± 0.72	0.73
	80	99.12± 0.13	0.13	99.05± 0.11	0.11
B	40	98.25± 0.55	0.56	98.31± 0.50	0.51
	60	98.44± 0.59	0.6	98.56± 0.61	0.62
	80	99.40± 0.62	0.62	99.50± 0.67	0.68
C	20	98.66± 0.86	0.87	98.71± 0.83	0.84
	30	98.82± 0.82	0.83	98.44± 1.13	1.14
	40	98.36± 0.14	0.14	98.68± 0.33	0.34

*(n=3)

Table 3: Precision Studies of Flecainide Acetate.

Ruggedness

Ruggedness of the proposed methods was determined for selected concentrations. The results were in the acceptable range for all the three methods and % RSD was found to be less than 2, indicating that the method is rugged. The detail results are tabulated as in Table 4.

Methods	Conc. (µg/ml)	Analyst I		Analyst II	
		% Amount found*± SD	% RSD	% Amount found*± SD	% RSD
A	60	99.07± 1.04	1.05	98.56± 0.84	0.85
B	60	98.54± 0.70	0.71	99.04± 1.05	1.06
C	30	98.86± 0.81	0.82	98.47± 0.83	0.84

*(n=6)

Table 4: Ruggedness Studies of Flecainide Acetate.

Repeatability

Repeatability was determined for selected concentrations of FA solution for six times and the % amount determined with % RSD less than 2 for all the three methods. The results are expressed in Table 5.

Methods	Amount taken (µg/ml)	Amount found*	% Amount found*± SD	% RSD
A	60	59.07	98.45± 0.87	0.88
B	60	59.14	98.56± 0.66	0.67
C	30	29.63	98.77± 0.84	0.85

Table 5: Repeatability Studies of Flecainide Acetate.

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Analysis of Flecainide Acetate in Bulk

The concentrations of the drug were calculated from linear regression equations. The % amount found was within 98.66% to 99.15% with % RSD less than 2 for all the three methods. The results are expressed in Table 6.

Methods	Amount taken (µg/ml)	Amount found*	% Amount found*± SD	% RSD
A	60	59.42	99.03± 1.03	1.04
B	60	59.49	99.15± 0.90	0.91
C	30	29.59	98.66± 0.99	1

*(n=6)

Table 6: Analysis of Flecainide Acetate in Bulk.

Analysis of Flecainide Acetate in Tablet Formulation

The spectrum was recorded at 296nm. The concentrations of the drug were calculated from linear regression equation. The % amount was found around 98% with % RSD less than 2 for all the three methods. The results are expressed in Table 7.

Methods	Amount taken (µg/ml)	Amount found*	% Amount found*± SD	% RSD
A	60	59.11	98.52± 0.80	0.81
B	60	59.2	98.67± 0.79	0.8
C	30	29.59	98.64± 0.68	0.69

*(n=6)

Table 7: Analysis of Flecainide Acetate in Tablet Formulation.

Sensitivity

The LOD and LOQ for selected drug were found to be 0.20 and 0.61µg, respectively, for method A, and 0.32 and 0.97µg, respectively for method B while it is found to be 0.03 and 0.12 for method C. The results are expressed in Table 8.

Methods	Linear Regression Equation	LOD (µg/ml)	LOQ (µg/ml)
A	$y = 0.0463x - 0.0403$ ($r^2 = 0.9995$)	0.2	0.61
B	$y = 0.0373x + 0.1652$ ($r^2 = 0.9993$)	0.32	0.97
C	$y = 327.29x + 5649.7$ ($r^2 = 0.9992$)	0.03	0.12

Table 8: Sensitivity Studies of Flecainide Acetate.

Conclusion

The spectrophotometric and chromatographic methods are developed and validated successfully for estimation of Flecainide acetate. The determination of drug candidate was done by taking bulk as well as in pharmaceutical tablet formulation. The results of the analysis of pharmaceutical formulation by the proposed methods are reproducible and reliable. This indicates that there is no interference of excipients. All these developed spectrophotometric and chromatographic methods are found to be simple, accurate, precise, and economical when validated as per ICH guidelines. Thus the proposed method can be used for routine analysis of Flecainide acetate.

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A review on in situ Nasal Gels for Nasal drug delivery system

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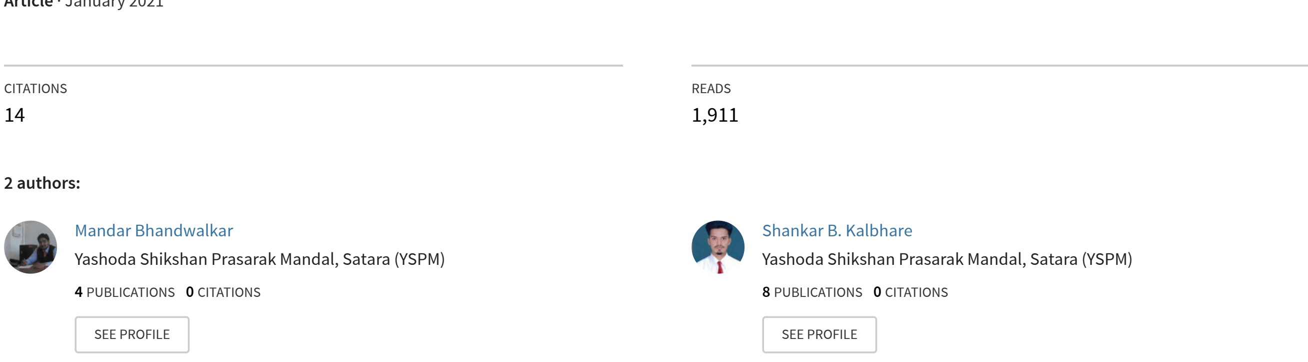

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REVIEW ARTICLE JPAR 2020

A review on *in situ* Nasal Gels for Nasal drug delivery system

Mandar J. Bhandwalkar*[1], Imran K Inamdar[1], Shankar B Kalbhare[1], Abhishek D. Changan[1], Supriya N Mandrupkar[2]

[1]Department of Pharmaceutics, YSPM's Yashoda Technical Campus, Satara - 415003, M.S., India.
[2]Krishna Institute of Medical Sciences, Karad, India.

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ABSTRACT: The oral route is the most favored technique for administering the drug orally in the body. As a result of certain limitations such as drug absorption, poor bioavailability, first-pass hepatic metabolism and drug targeting to particular organs, may cause problems for administration via oral route. Therefore parenteral route, transmucosal route and transdermal route are preferred over oral route. Intranasal route is deemed to be a desirable route because of the time profile of concentration a drug is close to that of the intravenous route. To increase patient safety and efficacy a new approach for drug delivery i.e. *in situ* nasal drug delivery system has been designed. In *in situ* nasal gels drug is administered as a low viscous solution. When in contact with nasal mucosa, the conformation of the polymer changes to gel form. The gel formulation via nasal route is appropriate for those drugs whose oral administration is problematic due to gastric discomfort, drug absorption, poor bioavailability of drug and first-pass hepatic metabolism. For the gel formulation various triggered polymers are used. The present review focused on therapeutic considerations, anatomy and physiology of nasal cavity, challenges and opportunities in nasal drug delivery, marketed products of *in situ* nasal gels and various evaluation parameters considered during preparation of in situ gel.

Corresponding author*
Prof. Mandar J. Bhandwalkar
Head of Department
Department of Pharmaceutics
YSPM's Yashoda Technical Campus
Satara- 415003, M.S., India.
Tel: +91-9028229028
Mail ID:mandar.j.bhandwalkar@gmail.com

Keywords: Nasal Anatomy, Drug delivery, *In situ*, Bioavailability, Transmucosal, Nasal gels.

INTRODUCTION:

Oral drug delivery is a most desirable route for drug administration. Whenever systemic effects are planned out, oral bioavailability of some compounds has promoted the search of more effective route for the systemic delivery [1].To attain faster and higher level of drug absorption nasal mucosa is the major route of administration in Transmucosal route of drug delivery [1]. Transmucosal nasal delivery has been a very promising route of delivery. Many drugs have been shown to achieve better systemic bioavailability through nasal route when compared with the oral route [2]. In the

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Ayurvedic systems of Indian medicines, nasal route there is a well-recognized form of treatment; it is known as NASYA KARMA. It is a convenient route for delivery of drugs, which are active in small doses and show minimal oral bioavailability. It is the most suitable dosage form for self-medication [2].

Intranasal administration represents a feasible choice for local and systemic delivery of various therapeutic compounds. The nasal mucosa has a large surface area that affords a quick onset of effects, potential for direct delivery to the central nervous system, it avoids first pass metabolism and shows non-invasiveness; all of this may maximize patient convenience, comfort and compliance [3]. The nasal mucosa acts as a permeation barrier to high molecular weight therapeutic compounds such as proteins and peptides. The tight junctions forming this barrier to paracellular drug delivery can be reversibly and safely opened. Intranasal treatment does not require sterile preparations, it is non-invasive, painless and can also be simply and readily administered by the patient, e.g. in an emergency [4-6].

Intranasal microemulsions, gels and microspheres have increased interest in current years to deliver protein and peptide through nasal route [2]. Recently, *in situ* gel has been introduced as a new dosage form in nasal drug delivery. Liquid nasal formulation compared with *in situ* gels is instilled as low viscosity solutions into the nasal cavity. On interaction with nasal mucosa, the polymer changes its conformation to a gel. So that it not only increases the contact time between drug and absorption site but also slowly releases drugs in the nasal cavity [7].

Therapeutic considerations:

Nose is an integral part of the body for inhalation purposes but when the nose is used as a drug delivery path the effective dose for different drugs has been achieved since the nose provides faster and higher levels of drug absorption and also self-administration possibilities. It is effective in delivery of local, systematic and central nervous system sites [8]. Therapeutic considerations are paramount when selecting the dosing route. These considerations include the pharmaceutical target like local versus systemic, the dosing frequency and the patient population. In approximately cases, intranasal delivery mostly preferred mode of administration [9].

Local delivery:

For the prevention of typical nasal disorder intranasal administration of medications is the natural choice. Common cases are corticoids and antihistamines for cold symptoms. In such a situation, the intranasal route is the principal choice for drug delivery since it shows a rapid sign of relief and shows lesser side effects [8].

Vaccine delivery:

The nasal mucosa was given some antigen through as a route of vaccination. Appropriate antigen with a good adjuvant to the nasal-associated with lymphoid tissue has the ability to encourage humoral and cellular immune responses [10]. This approach may be particularly effective for achieving rapid mass immunization, for instance in children and/or in developing countries and disaster areas [11]. The intranasal immunization can lead to the development of both local and systemic immunity.

Systemic delivery:

The intranasal administration is an effective way of delivering drugs systematically compared to oral and intravascular routes. Thus, the number of drugs administered as nasal formulations intended to attain systemic effects has widely improved [8].

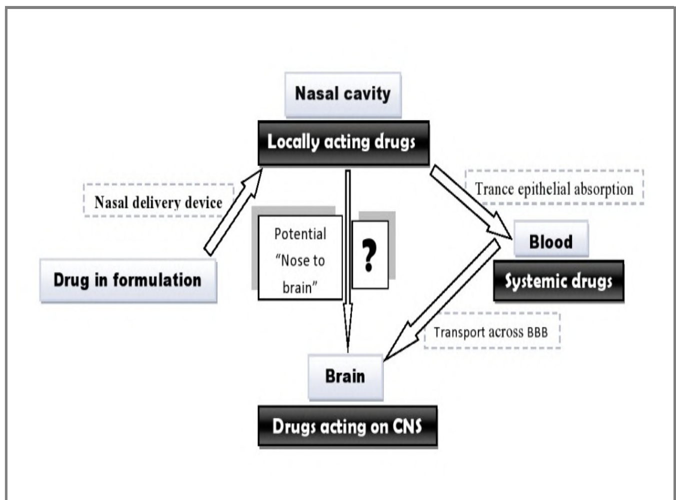

Fig 1. Nose to systemic circulation to brain pathway.

CNS delivery through nasal route:

The tight junctions of Blood Brain Barrier (BBB) surrounding the brain result in a greater trans-endothelial electric resistance (1500 to 2000 Ωcm^2) compared to that other tissues like skin, bladder, colon, lungs (3 to 33 Ωcm^2). The difficulty forced by those brain protective mechanisms has increased the interesting developing strategies to overcome them when brain drug exposure is required. The interpellation imposed by the interesting strategies to overcome them when brain drug exposure is needed for the mechanisms of brain defense has increased. In recent years, intranasal path has occurred

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as a positive approach for the delivery of drugs in the brain. The benefits of this drug delivery are the lack of gastrointestinal and hepatic pre-systemic removal [8].

Anatomy and physiology of nasal cavity:

The nasal cavity is divided into two hales by the nasal septum and extends back to the nasopharynx, while the nasal vestibule is the most anterior portion of the nasal cavity, opens up through the nostril to the nose (Fig 2). There are three main regions in the nasal cavity which are the nasal vestibule, the olfactory region and the respiratory region. The surface area in the nose can be increased by the lateral walls of the nasal cavity around 150 cm which contains a folded structure [12]. When compared with its minor volume, its surface area is very high. This folded structure involves three turbinates: the superior, median and the inferior. The nasal airway has narrow passages which is about 1 to 3 mm wide and which helps to perform its principal functions. The nasal cavity is covered by a mucous membrane that splits into two areas; non-olfactory and olfactory epithelium. In the non-olfactory region, the nasal vestibule is lined with skin like stratified squamous epithelial cells, whereas the respiratory field, having a standard airway epithelium filled with multiple microvilli, provides a large area accessible for drug absorption and transportation [13]. In this way, the position of the mucus layer from the anterior to the rare part of the nasal cavity is thus shifted. The mucous membrane protects the nasal turbinate and the atrium. The goblet cells secrete the mucus as mucus granules that swell in the nasal fluid to contribute to the mucus layer. The mucus secretion is composed of approximately 95 % water, 2 % mucin, 1 % salts, 1 % of other proteins such as albumin, immunoglobulin, lysozyme and lactoferrin and 1% lipids. The mucous secretion allows immune response suppression of inhaled bacteria and viruses [13].

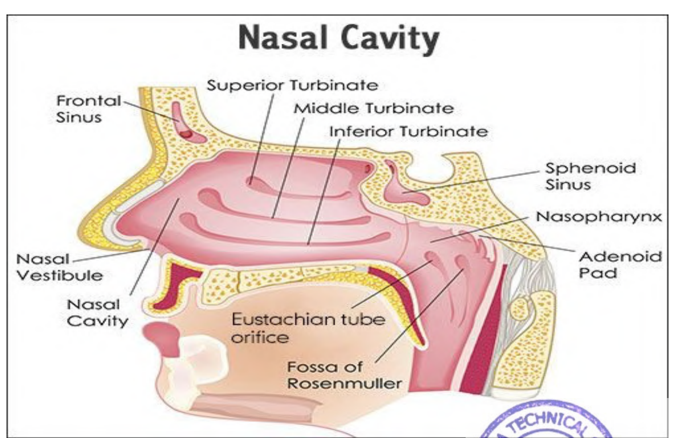

Fig 2. Anatomy of Nasal cavity.

It also performs a number of physiological functions as mentioned below.

- This forms the mucosa, and preserves this physically and enzymatically.
- The mucus has capacity to hold water.
- This exhibits electric behavior on the surface.
- It allows efficient heat transfer.
- It functions as glue and brings particulate matter to the nasopharynx.

Mechanism of drug absorption:

In the first step of absorption, the absorbed drug has to move from the nasal cavity to the mucus layer. Small uncharged drugs can pass through the mucus layer but greater and charged drugs find it very difficult to pass/cross it. Mucin is the main protein of mucus which has a tendency to bind to the solutes and hinders diffusion. The additional structural changes in the mucus layer occur as result of environmental (Temperature and pH) changes.

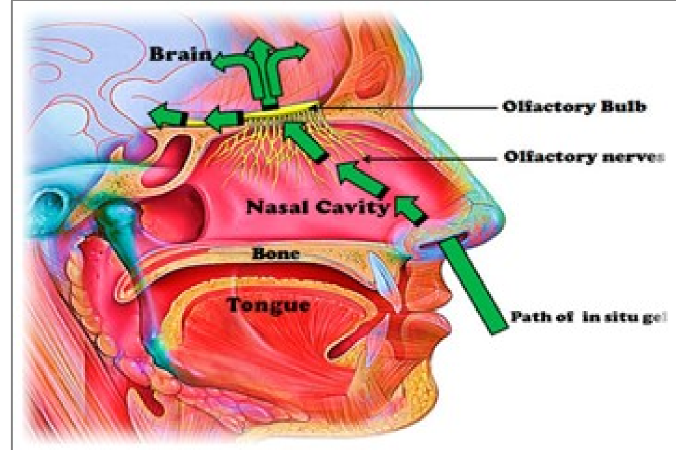

Fig 3A. Position of olfactory bulb with respect to brain and nasal cavity.

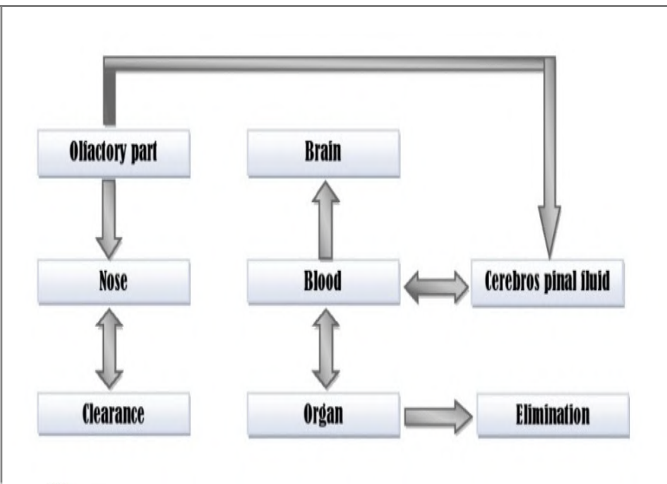

Fig 3B. Different routes to facilitate the drug entry into the brain after intranasal administration.

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In this process mainly two mechanisms have been predominantly observed, they are,

➢ Paracellular transport - It produces aqueous, but sluggish and passive transport route. This route is not appropriate for those drugs that have molecular weight larger than 1000 Dalton due to its poor bioavailability [14].

➢ Transcellular transport - This develops the lipoidal pathfor lipophilic drug transport [14].

Transport of the drug through the cell membrane can also be brought about by an active transport path. For example, chitosan, a natural biopolymer from shell fish opens tight junctions between epithelial cells to facilitate drug transport [14].

CHALLENGES AND OPPORTUNITIES FOR NASAL DELIVERY SYSTEMS:

The nasal delivery incentives cannot take full advantage of current nasal delivery systems, such as spray, pumps and pipettes. On the frontal section linked by skin a large portion of the dose is deposited and the deposited drug is not targeted for either topical site or systemic circulation. The patient acceptance is decreased because of the bad taste and discomfort to patients caused by drugs brought along the nose floor. Finally, a real task of prolonging nasal administration of drugs and vaccines is inadequate as a result of complex deposition in the remote region with sinus and middle ear openings and in the olfactory region. New advanced and costly drugs require reproducible bioavailability and demanding combination of dependable dosing and high patient compliance to confirm their efficacy and safety. Mostly liquid nasal products are delivered by metered spray pumps [15].

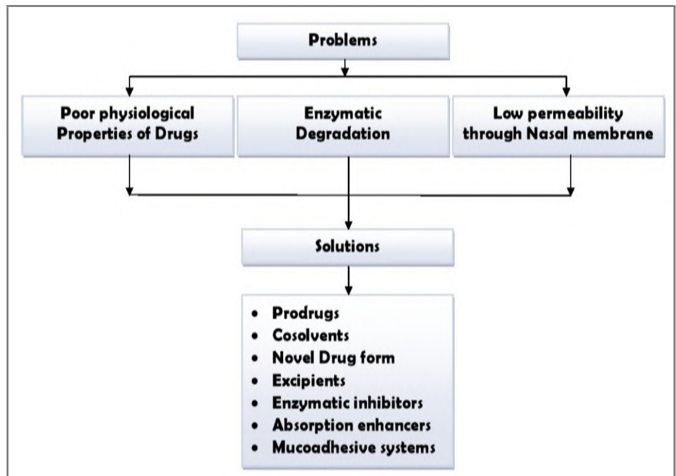

Fig 4. Problems and solutions for *in situ* nasal drug delivery system.

Problems and solutions for in- situ nasal drug delivery system:

The challenges in nasal drug delivery are improved physicochemical properties of drug and formulation, greater permeability and degradation of substances, modify nasal membrane, enhance drug residence time, decrease drug affinity to nasal enzymes, inhibit nasal enzymes, protect nasal enzymes against drug and reduced rapid mucociliary clearance.

CURRENT APPROACHES FOR NASAL PERMEATION ENHANCEMENT:

Based on low drug solubility, rapid enzymatic degradation in the nasal cavity, weak membrane permeation and rapid mucociliary clearance, the bioavailability of nasally administered drugs is largely limited. Several methods to overcome those limitations have been suggested. These methods are enlisted and described below.

Prodrugs:

Lipophilic drugs are poorly water soluble so they easily pass through biomembranes. To make the development of an aqueous nasal formulation probable with an appropriate concentration they should be administered as prodrugs with higher hydrophilic character [16]. The prodrugs must be converted rapidly to the parent drug when in the bloodstream. For example, in contrast with the parent drug many prodrugs of L-Dopa have higher solubility, hence allowing the progress of adequate nasal formulations [15-17].

Co-solvents:

In order to increase drug solubility the usage of co-solvents is an alternative approach for prodrugs. The co-solvents generally used in intranasal formulations include glycerol, ethanol, propylene glycol, polyethylene glycol, and they may be the most relevant as they are non-toxic, pharmaceutically safe, and non-irritating to nasal mucosa [15].

Enzymatic inhibitors:

Nasal mucosa layer serves as an enzymatic barrier during nasal drug delivery, because they have a wide range of enzymes. The different methods are used including protease and peptidase inhibitors to avoid enzymatic degradation. For example, amino peptidases are used as inhibitors in the degradation of calcitonin bestatine, comostate amylase and leupeptin and aprotinin as tyrosine inhibitors probably involved. Furthermore, to help prevent enzymatic degradation of drugs such as

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leucine encephalin, bacitracin, amastatin,boroleucin and puromycin has been used [18,19]. Eventually, the enzymatic reduction can also be done by using the absorption enhancers like (bile salts and fluidic acid). Disodium EDTA, an absorption enhancer, has been shown to limit enzymatic degradation of beta-sheet breaker peptide used to treat Alzheimer's disease [20].

Permeation enhancers:

Small and large hydrophilic drugs may exhibit poor permeability across nasal epithelium and may thereby show insufficient bioavailability. The permeation can be improved by administering in combination with absorption enhancers which make reversible changes to the epithelial barrier structure [21]. Permeation enhancers and their mechanism of action with examples are given in Table 1.

METHODS OF FORMULATION OF *IN SITU* NASAL GEL:

Cold method:

In this method of formulation, the product and sample quantity of double distilled water are mixed in a refrigerator and held overnight at 4 ºC. Then the in situ gelling polymer is further added slowly with constant stirring.

In a refrigerator the dispersion is stored till a clear solution is designed and volumes adjusted. When gelling polymers like poloxamer, chitosan or Carbopol are used for formulation then this method is selected. In view of the fact that polymeric dispersion of poloxamer persists as a solution at lower temperatures and is concentrated in gel at higher nasal temperatures because the solubility of poloxamer's propylene oxide chain decreases at high temperatures, resulting in precipitation or salting from polymer.

Likewise, chitosan often requires low temperatures to survive as a solution at room temperature, its hydrophobicity increases with higher temperatures [22].

Hot Method:

This form is preferred if gellan gum or pectin is used as a gelling polymer. Gellan chains dissolve in water at higher temperatures and postulate a random coil conformation with high segmental mobility at high temperatures and proceed as a solution at higher temperatures. In the presence of ions such as K^+ or Ca^{2+} sol-gel transformation occurs when gellan gum solution is cooled. Similarly, pectin also needs a higher temperature for demethoxylation purposes which helps to formulate a solution or dissolve pectin [23].

TRIGGERED *IN SITU* GELLING FORMATION:

Temperature triggered *in situ* gel:

There are some polymers which undergo large and unexpected physical and chemical changes in response to small external changes in their environmental conditions. Such polymers are called Stimuli-responsive polymers. They are also called as stimuli-sensitive, intelligent, smart or environmentally sensitive polymers. These polymers recognize a stimulus as a signal, judge the degree of the signal and then transform their chain conformation in response.

Temperature sensitive polymers are the most extensively studied class of environmentally responsive polymer systems in drug delivery. This is because temperature is relatively easy to control and also easily applicable to both *in vitro* and *in vivo*.

In this system, gelling of solution is triggered by alteration in temperature, thus sustaining the drug release.

These hydrogels exist in liquid form at room temperature (20 to 25°C) and undergo gelation when comes in contact with body fluid (35 to 37°C). The use of biomaterial whose transition from sol-gel is triggered by increase in temperature is an attractive way to approach *in situ* formation. The best critical temperature range for such systems is ambient and physiologic temperature; such that clinical manipulation is facilitated and no external source of heat other than that of the body is required to trigger gelation.

pH triggered *in situ* gel:

Another physiological stimulus that induces formation of *in situ* gel is pH. Polymers included in this class contain an acidic or a basic group that either accept or release protons when they are exposed to different environmental pH. Hence these are called pH sensitive polymers. Most of the pH sensitive polymers containing anionic group are based on PAA (Carbopol®, Carbomer) and its derivatives [7].

Ion- activated *in situ* gel:

In this type of gelation, a polymer that undergoes phase transition in presence of ions. Gellan gum is an anionic polysaccharide that undergoes phase transition in the presence of monovalent and divalent cations like Ca^{2+}, Mg^{2+}, K^+, and Na^+ present in the nasal secretion.

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Table 1. Mucosal penetration enhancers and mechanisms of action with examples.

Classification	Examples	Mechanism
Surfactants	Anionic: Sodium lauryl sulphate Cationic: Cetyl pyridinium Chloride. Nonionic: Poloxamer, Span	Intercellular lipid disease, Protein domain integrity, Distorts membrane
Bile salts	Sodium glycodeoxycholate, Sodium glycocholate, Sodium taurodeoxycholate	Mucolytic activity, Open tight junctions, Distorts membrane
Cyclodextrins	α,β,γ - Cyclodextrin, Methylated β - Cyclodextrins	Inclusion of membrane compounds, Open Tight junctions
Fatty acids	Oleic acid, Methyloleate, Lauric acid, Caprylic acid, Phosphotidylcholine.	Phospholipid fluidity improves domain, Distorts membrane.
Cationic compounds	Poly-L-arginine, L-lysine	Ionic interaction on the mucosal surface with negative charge
Chelators	Sodium salicylate, EDTA, Sodium citrate, citric acid	Interfere with CaPolyacrylates
Positive charged polymers	Chitosan, Trimethylchitosan	Ionic interaction with negative chargeon the mucosal surface
Bioadhesive Materials	Carbopol, Chitosan Starch	Opens tight junctions, Reduce nasal clearance

Table 2. Delivery System Based Approaches for Intranasal Drug Delivery.

Formulation	Advantages	Disadvantages
Nasal spray	Nasal sprays may be formulated in the form of solution and suspension Exact dose can be delivered via metered dose pumps and actuators	Less efficient than nasal drops when human serum albumin is stored in the nostrils
Nasal drops	Simple and convenient system	Lack of dose precision
Nasal gels	Due to high viscosity reduction of post nasal drip, reducing the effect of tastes due to reduced swallowing and reduction of anterior formulant leakage	Local side effects
Nasal powders	Absence of preservatives and superior stability.	The appropriateness of powder composition depends on the solubility, particle size, aerodynamic properties and nasal discomfort of active drugs
Liposomes	Active encapsulation of large and small molecules with high hydrophilicity and pKa values	Production cost is high Short half-life
Nanoparticles	Deposits their small size	Only the smallest nanoparticles penetrate to the mucous membrane by paracellular route and in a limited amount

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POLYMERS USED FOR THE *IN SITU* GELLING SYSTEM PREPARATION:

Polymer used for pH *In situ* gelling system:

***Carbopol*:**

The water absorption property of Carbopol polymers is very good. Because of the pKa of that polymers is 6.0, they swell in water up to 1000 times its original volume adds 10 times its original diameter to form a gel until exposed to a pH of 4.0 to 6.0. Carbopol polymer has high molecular weight and cross linked polyacrylic acid derivatives and also it has strong mucoadhesive properties. It will reduce polymer concentration and improve concentration and improve gelling property when cellulose addition is done. The mostly used gelling polymers are Carbopol 934 and Carbopol 841 [24].

The Mucoadhesive property is due to electrostatic interaction or hydrophobic interaction, hydrogen bonding. It is an acidic molecule. The carboxylic group within the molecule partially dissociates and forms a spiral when dispersed in water. As it is pH sensitive polymer, a rise in the pH of the solution results in polymer swelling. In two stages the gelling effect is activated, neutralization of result by addition of sodium hydroxide or potassium hydroxide, triethanolamine [24].

Polymer used in temperature sensitive *In situ* gelling system:

***Poloxamer*:**

It is a water soluble tri-block copolymer. It contains two polyethylene oxide and polypropylene oxide in an ABA configuration [25].

It has excellent thermal setting properties and improved drug resistance time and it is also known as Pluronic. It is used as a gelling agent and solubilizing agent. It gives colorless, transparent gel. It is available innumerous molecular weights, having different gelling properties. The gelling property depends upon the ratio and partition of the hydrophobic and hydrophilic chain.

The Poloxamer consists of essential polypropylene oxide enclosed by polyethylene oxide. At room temperature (25 °C), it works as viscous liquid and when temperature increases (37 °C) it transforms to transparent gel. It forms a small micellar subunit in solution at low temperatures and changes in temperature results in increased viscosity resulting in swelling to form a large micellar cross-linking network [26,27].

Polymer used in ion sensitive *In situ* gelling system:

***Sodium alginate*:**

It is extracted from brown algae and it is salt of alginic acid. It is linear block polysaccharides. It consists of two types of monomers β-D-Mannuronic acid and α-L glucuronic acid residues linked by 1,4-glycosidic linkages.It is non-toxic and biodegradable. Due to its carboxylic group it has good mucoadhesive property.

The monomers of alginate β-D-Mannuronic acid and α-L glucuronic acid are arranged as M-M block with altering sequence (M-G) block.G-block polymer interacts with calcium molecules resulting in homogenous gel formation. Mechanical strength and porosity of hydrogel depends on the ratio G: M, type of cross linker used [24].

Synthetic Polymers:

***N-isopropyl acrylamide copolymers*:**

It is a non-biodegradable LCST polymer, which collapses about 32 °C in water and forms cross-linked gels.

***PEG/ PLGA Block copolymers*:**

This is a new concept because they combine thermal gelation, biodegradable, and no toxicity. It was planned for an injectable gel device with greater safety and longer gel length [28].

EVALUATION OF NASAL *IN SITU* GELS [26-32]:

Clarity:

The visual inspection under a black and white backdrop will assess the clarity.

Viscosity:

The viscosity and rheological properties of the polymer formulation can be calculated in solution or gel made from artificial tissue fluid and with various viscometers such as the Brookfield viscometer, cone and plate viscometer.

Texture analysis:

For main indications of the syringe capacity of sol the firmness, uniformity and cohesiveness of formulation may be determined using a texture analyzer so the preparations can easily administer *in-vivo*.

Drug content:

About 1ml of prepared solution is taken in 10ml volumetric flask and made up to 10ml and then diluted with 10ml of distilled water. About 1ml from this solution again diluted up to 10ml with distilled water. Using the UV visible spectroscopy, formulated solutions at specific wavelengths are tested.

Gel strength:

Gel strength can be measured by using a Rheometer. A specific volume of gel is prepared in a beaker. A probe is

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pushed through the gel. The changes in the load on the probe can be determined as a function of the depth of immersion of the probe below the gel surface.

Sol-gel transition temperature and gelling time:

The temperature and pH of the sol-gel process should be measured for *in situ* gel forming systems. Gelling time is the time required to detect *in situ* gelling in the first place. The thermosensitive *in situ* gel must be tested for in situ gelling at body temperature.

Drug polymer interaction study and thermal analysis:

By using Fourier Transform Infrared (FTIR) Spectroscopy interaction study may be determined. The technique of employing KBr pellet method the nature of the interacting forces may be measured. The Thermo Gravimetric Analysis (TGA) for *in situ* formation method can be performed to measure the percentage of water in hydro gel. The Differential Scanning Calorimeter (DSC) used to detect some difference in thermo gram compared to the pure active ingredients used for gelation.

Gelling capacity:

Mix *in situ* gel with simulated tear fluid (in the proportion of 25: 7, i.e. application volume 25 µL and volume of tear fluid in eye is 7 µL) to find out the gelling capacity of ophthalmic products. The gelation may be assessed visually by noting the time for and time taken for dissolution of the formed gel.

Sterility testing:

As per the IP 1996 the sterility testing is carried out. In this testing, incubate the formulation in the fluid thioglycollate medium at 30 to 35 ºC for a period of 14 days to find the growth of bacteria and in the soybean casein digest medium at 200 to 25 ºC to find the growth of the fungi in the formulation.

Accelerated stability studies:

In amber colored vials and sealed with aluminum fail the formulation is replaced for the short term. As per ICH state guidelines the accelerated stability done at 40±20 °C and 75±5 % RH.

***In vitro* drug release study:**

In situ preparations to be given by the nasal, ocular, the drug release tests are carried out using the plastic dialysis cell. The cell consists of two half cells containing a donor compartment and a receptor compartment. These are isolated by cellulose membranes. The preparation sol form is put inside the donor container. In an incubator, the assembled cell is then shaken horizontally. The total volume of the receiver solution can be removed at intervals and replaced with the fresh media. This receptor solution is examined using analytical receptor media and placed in a shaker water bath at appropriate temperature and oscillation rate. Samples are routinely removed and examined.

APPLICATION OF *IN SITU* DRUG DELIVERY SYSTEM:

Oral drug delivery system:

The natural polymers including pectin, xyloglucan and gellan gum are used in situ forming oral drug delivery systems. The gelation of pectin usually occurs in the presence of H^+ ions, a source of divalent ions, typically calcium ions are required to produce the gels used as vehicles for drug delivery. The paracetamol was reported as an oral in situ gelling pectin formulation for sustained delivery as possible [33].

Ocular drug delivery system:

The natural polymers such as gellan gum, alginic acid and xyloglucan are mostly used in situ forming oral drug delivery systems. The various compounds such as antimicrobials, anti-inflammatory agents and autonomic drug mostly used to alleviate intraocular stress in glaucoma for this purpose have been used for the local delivery of ophthalmic drugs. Due to the high tear fluids and the complexities, the substances are quickly removed from the eyes. The conventional delivery systems often lead to poor bioavailability and therapeutic reactions. So, bioavailability problems have been identified in ophthalmics in situ gels. Because of the temperature and ionic concentration (Ca^{++}) in the tear fluid, gellan's aqueous solution lowered into the eye undertakes a transition to gel state. In the pharmaceutical application the interest about the gellan gum has focused on its application for ophthalmic drug delivery. The drug release from these in situ gels is delayed due to longer precorneal contact times of the viscous gels compared with conventional eye drops [34].

Nasal drug delivery system

The *in situ* gel device for the nasal delivery of mometasone furoate was developed and tested for its safety and efficacy in the treatment of allergic rhinitis by choosing the nasal route. The polymers such as xanthan gum and gellan gum have been used as *in situ* gel

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Table 3. Marketed products of Oral floating *in situ* gels [38].

Drug substances	Brand Name	Indication	Dosage form	Manufacturer
Levodopa, Benserazide	Modapar	Indicated for the prevention of Parkinson's disease	Floating capsule	Roche Products, USA
Diazepam	Valrelease	Indicated to treat management of anxiety disorders	Floating capsule	Hoffmann-LaRoche, USA
Aluminium hydroxide, Magnesium carbonate	Liquid Gaviscon	Indicated to treat the symptoms of too much stomach acid such as stomach upset, heartburn and acid indigestion	Effervescent Floating Liquid Alginate Preparation	Glaxo Smith Kline, INDIA
Aluminium Magnesium antacid	Topsalkan	Indicated for the prevention of relieve heartburn, acid indigestion, and upset stomach	Floating Liquid alginate Preparation	Pierre Fabre Drug, France
Ferrous sulphate	Conviron	Used in the treatment of megaloblastic anemia's, infancy, pregnancy, anemias of nutritional origin etc.	Colloidal gel forming FDDS	Ranbaxy, INDIA
Ciprofloxacin	Cifran OD	Used in the treatment of Pneumonia, Bronchitis, Gonococcal infection, joint infection	G; 2as-generating floating tablets	Ranbaxy, INDIA

Table 4. Marketed products of ophthalmic *in situ* gels [34].

Drug substances	Brand Name	Indication	Dosage form	Manufacturer
Timolol maleate	Timoptic-XE	Indicated in the prevention of raised intraocular pressure in patients with open ocular hypertension or angle glaucoma	Solution	Merck and Co. Inc
Azithro-mycin	Azasite	Indicated for the prevention of bacteria caused by sensitive conjunctivitis isolated of some microorganism i.e. Haemophilus influenza, Staphylococcus aureus, streptococcus mitis group, streptococcus pneumoniae	Solution	In-Site Vision
Lidocaine hydrocloride	Akten TM	Suitable for treating eye surface anesthetics during ophthalmological procedures	Gel	Akten
Ganciclovir	Virgan	Used to treat cytomegalovirus disease in solid organ transplant recipients and in individuals	Gel	Spectrum Thea Pharmaceuticals
Pilocarpine hydrocloride	Pilopine HS	Used to minimize the pressure InSitethe eye and treat dry mouth	Gel	Alcon Lab. Inc.

Table 5. Marketed products of Nasal *in situ* gels [39].

Drug substances	Brand Name	Indication	Dosage form	Manufacturer
Fluconazole	Diflucan	Used to prevent the Antifungal infections	Solution (Spray)	Pfizer Limited, India
Zinc gluconate, Zinc acetate	Zicam	Used to prevent cold and the relief of cold symptoms such as sore throat, runny nose, cough and congestion	Solution (Spray)	Matrixx Initiatives, Inc

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Tables 6. Marketed products of Rectal and Vaginal *in situ* gels[40].

Drug substances	Brand Name	Indication	Dosage form	Manufacturer
Diazepam	Diastat	Used to prevent a range of conditions, including alcohol withdrawal syndrome, anxiety, benzodiazepine withdrawal syndrome, seizures, muscle spasms, restless legs syndrome and trouble sleeping	Gel	Valeant Pharmaceuticals
Dinoprostin	Prostin E	Used in labor induction, termination of pregnancy, bleeding after delivery and in newborn babies to keep the duct us arteries open	Suppository	Pfizer Limited, India
Metronidazole	Metrogel Vaginal	Used to prevent certain types of bacterial infections in the vagina	Gel	JM Pharmaceuticals
Progesterone	Crinone	Used to prevent gynecological disorders	Gel	Watson Pharma, Inc.

Table 7. Marketed products of injectable *in situ* gels [41].

Drug substances	Brand Name	Indication	Dosage form	Manufacturer
Ganciclovir	Vitrasert	Used to prevent cytomegalovirus infections	In situ gel	Bausch Health Companies Inc.
Doxycycline	Atridox	Used to prevent adult gum disease (periodontitis)	Gel	DenMat
Leuprolide acetate	Eligard	Used to prevent breast cancer,endometriosis, prostate cancer, uterine fibroids, and primary puberty	Injectable suspension	Tolmar Pharmaceuticals

forming systems. The animal studies were conducted using an allergic rhinitis model and the results of in situ gel on antigen mediated nasal symptoms have been observed in sensitized rats. As compared to marketed nasonex formulation (mometasone furoate suspension 0.05 %) *in situ* gel was found to inhibit the increase in nasal symptoms [35].

Rectal drug delivery system:

It also possesses a potential application for rectal and vaginal drug delivery in situ gels. Miyazaki et al. researched the use of xyloglucan based thermoreversible gels for the rectal drug delivery of indomethacin [19].

Vaginal drug delivery system:

A mucoadhesive, thermosensitive, prolonged release vaginal gel incorporating clotrimazole-β-cyclodextrin complex was prepared for the treatment of vaginitis and gives better therapeutic effectiveness and patient compliance. Pluronic F-127 was used as an *in situ* gel forming polymer with mucoadhesive polymers such as Carbopol 934 and hydroxyl propyl methyl cellulose to ensure long residence time at the application site [36].

Injectable drug delivery system:

For tumor treatment an injectable, novel, thermosensitive *in situ* gelling hydrogel was developed. It consists of a chitosan solution filled with drugs neutralized with β-glycerophosphate [37].

MARKETED IN SITU GEL FORMULATIONS:

The marketed *in situ* gel formulations are enlisted in Table 3, 4 and 5.

CONCLUSION:

Nasal drug delivery is a novel platform and it is a promising alternative to injectable route of administration. There is possibility in the near future that more drugs will come in the market in the

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form of nasal formulation intended for systemic treatment.

Development of a drug with a drug delivery system is influenced by several factors. For the treatment of long illnesses such as diabetics, osteoporosis, fertility treatment novel nasal products are also expected to be marketed. Bioavailability of nasal drug products is one of the major challenges in the nasal product development. In contrast, a huge amount of money is investigated by pharmaceutical companies in the development of nasal products, because of growing demand of nasal drug products in global pharmaceutical market. So for the avoidance of side effect and improve effectiveness of nasal products we should pay attention to basic research.

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CONSUMERS VIEW ON SAFETY OF OVER THE COUNTER DRUGS PREFERRED AT RETAILERS AND INFORMATION SOURCES IN (WADHE) SATARA REGION

[1]*Prathamesh B. Yarsanwar, [2]Karishma J. Baid, [3]Shankar Balu Kalbhare and [4]Mandar J. Bhandwalkar

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***Corresponding Author**
Prathamesh B. Yarsanwar
YSPM'S Ytc Fop Satara.

ABSTRACT

Resources for all stakeholders but can be harmful for consumers. Creating awareness of rational drug use is only possible through continued public education with a broad vision of good health and wellbeing of the society. In developed economies, the four As of marketing has been addressed fairly well but in India, the accessibility and awareness is still on a lower side especially for allopathic OTC drugs. In India, the import, manufacture, distribution and sale of drugs and cosmetics are regulated by the Drugs and Cosmetics Act (DCA) and its subordinate legislation, the Drugs and Cosmetics Rules (DCR).

KEYWORDS: OTCs, distribution of patient, side effect of drug, Direction on package, dose regime, survey and Questionnaire, Satara.

INTRODUCTION

Over-the-counter (OTC) medications drugs available to consumers without a prescription play an increasingly vital role in our healthcare system and are the most prevalent means of treating the majority of common health problems in the United States.[1] There are over 80 therapeutic categories of OTC drugs which can be grouped in 12 broad therapeutic classes.[2] Over-the-counter (OTC) drugs are medicines that may be sold directly to a consumer without a prescription from a healthcare professional, as compared to prescription drugs, which may be sold only to consumers possessing a valid prescription. In many countries, OTC drugs are selected by a regulatory agency to ensure that they are ingredients that are safe and effective when used without a physician's care. OTC drugs are usually regulated by active

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pharmaceutical ingredients (APIs), not final products. By regulating APIs instead of specific drug formulations, governments allow manufacturers freedom to formulate ingredients, or combinations of ingredients, into proprietary mixtures.[3,4]

OTC Medications

Analgesics and antipyretics :- Paracetamol, Diclofenac
Cold, cough, and allergy products:- Levocetrizine,Cetirizine
Gastrointestinal products:- Omiprazole, Ranitidine
Dermatological products:-Dermi5, Dermiside
antifungals, :- Flucanazole, Itraconazole,
Ophthalmic products:-Ketotifen, Nepafenac
Oral health care products: Mouthwash, ToothPest
Menstrual products:- Mefenamic acid,
Vitamins Product :- Cholecalciserol, Ferrous gluconate

Paracetamol

Paracetamol, also known as acetaminophen or APAP, is a medication used to treat pain and fever. It is typically used for mild to moderate pain relief. Evidence for its use to relieve fever in children is mixed. It is often sold in combination with other ingredients such as in many cold medications. In combination with pain medication, paracetamol is also used for more severe pain such as cancer pain and pain after surgery. It is typically used either by mouth or rectally but is also available intravenously. Effects last between two and four hours.[5]

Paracetamol is generally safe at recommended doses. Serious skin rashes may rarely occur, and too high a dose can result in liver failure. It appears to be safe during pregnancy and when breast feeding. In those with liver disease, it may still be used, but in lower doses. Paracetamol is classified as a mild analgesic. It does not have significant anti-inflammatory activity and how it works is not entirely clear.[6]

Omeprazole

Omeprazole contains a tricoordinatedsulfinylsulfur in a pyramidal structure and therefore can exist as either the (*S*) - or (*R*)-enantiomers. Omeprazole is a race mate, an equal mixture of the two. In the acidic conditions of the canaliculi of parietal cells, both enantiomers are converted to chiral products.[7] (sulfenicacidand sulfenamide configurations) which react with

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a cysteine group in H^+/K^+ ATPase, thereby inhibiting the ability of the parietal cells to produce acid. AstraZeneca has also developed esomeprazole (Maximum) which is a eutomer, purely the (*S*)-enantiomer, rather than a race mate like omeprazole.[8]

Fluconazole

Fluconazole is an antifungal medication used for a number of fungal infections. This includes candidiasis, blast mycosis, coccidiodomycosis, cryptococcosis, histoplasmosis, dermatophytosis, and pityriasisversi colour. It is also used to prevent candidiasis in those who are at high risk such as following organ transplantation, low birth weight babies, and those with low blood neutrophil counts. It is given either by mouth or by injection into a vein. Common side effects include vomiting, diarrhoea, rash, and increased liver enzymes. Serious side effects may include liver problems, QT prolongation, and seizures. Fluconazole was patented in 1981 and came into commercial use in 1988.[9,10] It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system. Fluconazole is available as a generic medication. The wholesale cost in the developing world is about 0.05 to 0.10 USD per day. In the United States the wholesale price is about 1.14 to 1.75 USD per day as of 2016.[11]

Levocetirizine

Levocetirizine (as levocetirizine dihydrochloride) is a third-generation, non-sedating antihistamine, developed from the second-generation antihistamine cetirizine. Chemically, levocetirizine is simply the isolated levorotary enantiomer of cetirizine, which is sold as a racemic mixture.[12]

Erythromycin

Erythromycin is an antibiotic useful for the treatment of a number of infections. This includes respiratory tract infections, skin infections, chlamydia infections, pelvic inflammatory disease, and syphilis. It may also be used during pregnancy to prevent Group B streptococcal infection in the new-born. Erythromycin may be used to improve delayed stomach emptying. It can be given intravenously and by mouth. An eye ointment is routinely recommended after delivery to prevent eye infections in the new-born.[13,14]

Diclofenac

The primary mechanism responsible for its anti-inflammatory, antipyretic, and analgesic action is thought to be inhibition of prostaglandin synthesis by inhibition of the transiently

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expressed prostaglandin-endoperoxide synthase-2 (PGES-2) also known as Cycloxygenase-2 (COX-2). It also appears to exhibit bacteriostatic activity by inhibiting bacterial DNA synthesis.[15]

Inhibition of prostaglandin synthesis occurs systemically resulting undesirable symptoms such as irritation of the gastric epithelium. This is the main side effect of diclofenac. Diclofenac has a low to moderate preference to block the constitutively expressed COX-1 isoenzyme (approximately 10-fold) and is said to have, therefore, a somewhat lower incidence of gastrointestinal complaints than noted with aspirin which irreversibly inhibits COX-1.[16]

Nimesulide

Nimesulide is a nonsteroidal anti-inflammatory drug (NSAID) with pain medication and fever reducing properties. Its approved indications are the treatment of acute pain, the symptomatic treatment of osteoarthritis, and primary dysmenorrhoea in adolescents and adults above 12 years old.

Side effects may include liver problems, it has a multifactorial mode of action and is characterized by a fast onset of action. It works by blocking the production of prostaglandins (a chemical associated with pain), thereby relieving pain and inflammation.[17]

Clotrimazole

Clotrimazole, sold under the brand name Canesten among others, is an antifungal medication. It is used to treat vaginal yeast infections, oral thrush, diaper rash, pityriasis versicolor, and types of ringworm including athlete's footand jock itch.It can be taken by mouth or applied as a cream to the skin or in the vagina.[18]

Albendazole

Albendazole, also known as albendazolum, is a medication used for the treatment of a variety of parasitic worminfestations.[19]

Common side effects include nausea, abdominal pains, and headaches. Potentially serious side effects include bone marrow suppression which usually improves on stopping the medication. Liver inflammation has been reported and those with prior liver problems are at greater risk. It is pregnancy category C in the United States and category D in Australia,

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meaning it may cause harm if taken by pregnant women. Albendazole is a broad-spectrum anthelminthic agent of the Benzimidazole type.[20]

Loperamide

Loperamide, sold under the brand name Imodium among others, is a medication used to decrease the frequency of diarrhoea. It is often used for this purpose in gastroenteritis, inflammatory bowel disease, and short bowel syndrome.]It is not recommended for those with blood in the stool. The medication is taken by mouth.[21]

Common side effects include abdominal pain, constipation, sleepiness, vomiting, and a dry mouth. It may increase the risk of toxic mega colon. Loperamide's safety in pregnancy is unclear, but there is no evidence of harm. It appears to be safe in breastfeeding. It is an opioid with no significant absorption from the gut and does not cross the blood brain barrier when used at normal doses. It works by slowing the contractions of the intestines.[22]

Plan of Work

Collection of information about OTCs taken by patients in satara. Gathering useful and relevant information that is essential for careful consideration to the design of questionnaire. Prepare questionnaire for patients. Collection of answers to the questionnaire by personal visit to patients.

Questionnaire Method Was Followed

A questionnaire is a research instrument consisting of a series of questions and other prompts for the purpose of gathering information from respondents. Questionnaires have advantages over some other types of surveys in that they are cheap, do not require as much effort from the questioner as verbal or telephone surveys, and often have standardized answers that make it simple to compile data.

According to questions

1. Distribution of patient according to Age in year
2. Distribution of patient according to Sex
3. Distribution of patient according to education
4. On whom will you Trust while taking OTC drug
5. Patient experiencing any Side effect of drug
6. Patient following dose regime
7. Which information sources used by patient?

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8. Which formulation of OTC drugs Preferred?

Basic Rules for Questionnaire Item Construction

Use statements which are interpreted in the same way by members of different subpopulations of the population of interest. Use statements where persons that have different opinions or traits will give different answers. Think of having an "open" answer category after a list of possible answers. Use only one aspect of the construct you are interested in per item. Use positive statements and avoid negatives or double negatives. Do not make assumptions about the respondent. Use clear and comprehensible wording, easily understandable for all educational levels. Use correct spelling, grammar and punctuation.

Information sources

1. Advertisement in TV shows
2. Advertisement in Newspaper
3. Previous Prescription
4. Marketing people
5. Pharmacist(preferred)
6. Nursing home
7. Government publications
8. Relatives, Friends, Family members
9. Magazine
10. Films
11. Photograph
12. Radio
13. Speeches
14. Internet
15. Flax
16. Health Camp

List of drugs

Sr.No	List of drugs	Brand name	Dose	Price per strip
1	Paracetamol	Crosine	BD	13
2	Omiprazole	Omee-D	OD	51
3	Nimesulide	Nicip	BD	36
4	Erthromycine	Erthromycine-150	BD	95
5	Itracanazole	Itrafix-200	OD	100
6	Levocetrizine	Okacet	BD	16
7	Flucanazole	Fluca-150	OD	12

Table 1: Distribution of patient according to Age in years.

Limit	Total number of cases	Respondents (%)
18 -29	60	17.14%
30-49	100	28.57%
50-69	150	42.85%
70<	40	11.42%

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Table 2: Distribution of patient according to Sex.

Gender	Total number of cases	Respondents (%)
Male	225	64.28%
Female	125	35.71%

Table 3: Distribution of patient according to education.

Education	Total number of cases	Respondents (%)
Government / service	70	20%
Private / business	120	34.28%
Retired	48	13.71%
Student	67	19.14%
Unemployed	45	12.85%

Table 4: On whom will you Trust while taking OTC drug.

Patient Response	Total number of cases	Respondents (%)
Doctor	95	27.14%
Pharmacist	160	45.71%
Friends	56	16.00%

Table 5: Patient experiencing Side effect of drug.

Patient Response	Total number of cases	Respondents (%)
Yes	46	13.14%
No	304	86.85%

Table 6: Patient following dose regime.

Patient Response	Total number of cases	Respondents (%)
Yes	286	81.71%
NO	64	18.28%

Table 7: Which information sources used by patient?

Sr. No.	Sources of information	Number of patient	Respondents (%)
1	Advertisement in TV shows	142	40.57%
2	Advertisement in News paper	78	22.28%
3	Previous Prescription	43	12.28%
4	Social media like- Whats app, Facebook, Gmail etc.	67	19.14%
5	Relatives, Friends, Family members.	70	20%

Table 8: Which formulation of OTC drugs Preferred?

Sr. No	Formulation	Total number of cases	Respondents%
1	Tablet	146	41.71%
2	Liquid	74	21.14%
3	Lotion	32	9.14%

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4	Powder	47	13.42%
5	Creams	51	14.57%

Charts

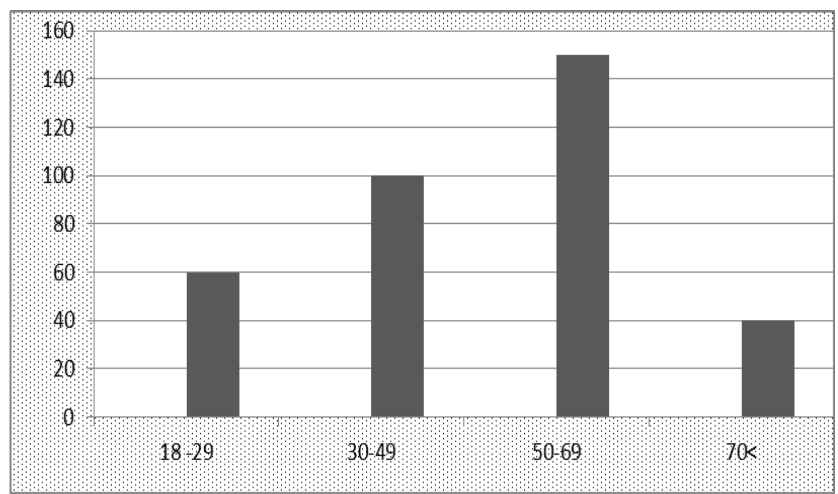

Chart: 1: Distribution of patient according to Age in years.

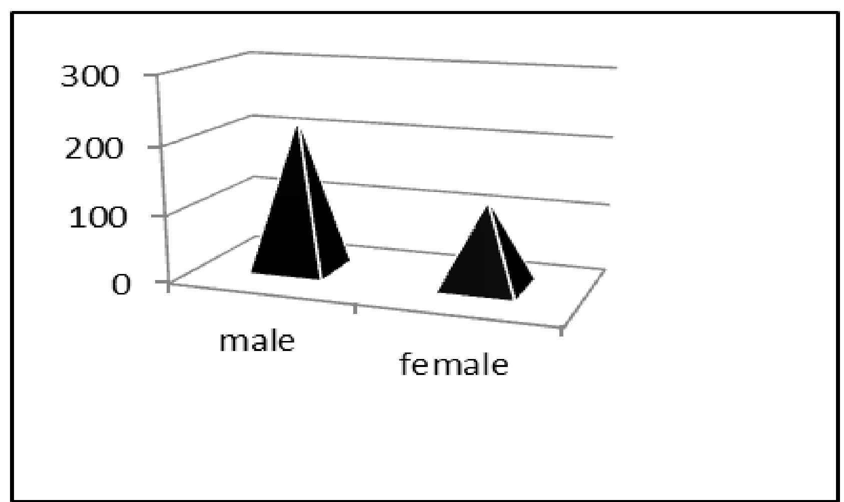

Chart 2: Distribution of patient according to Sex.

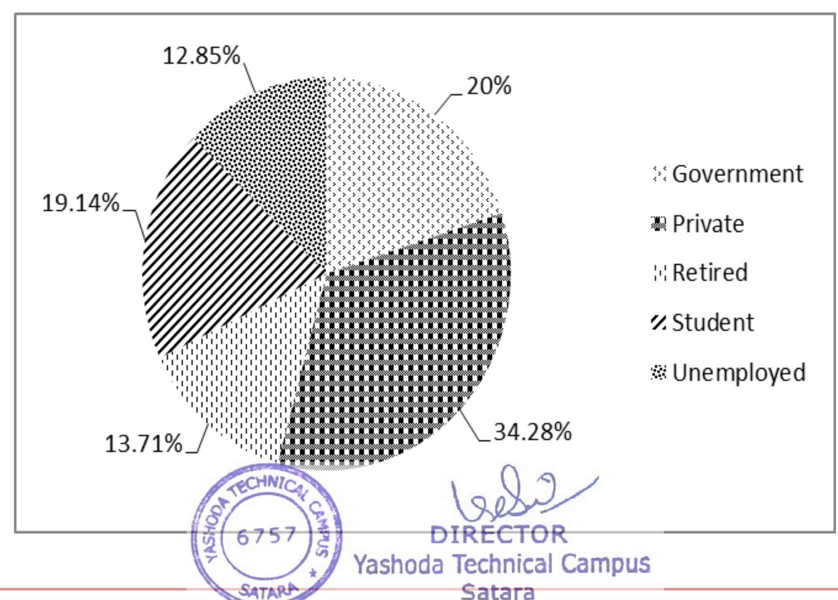

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Chart: 3: distribution of patient according to education.

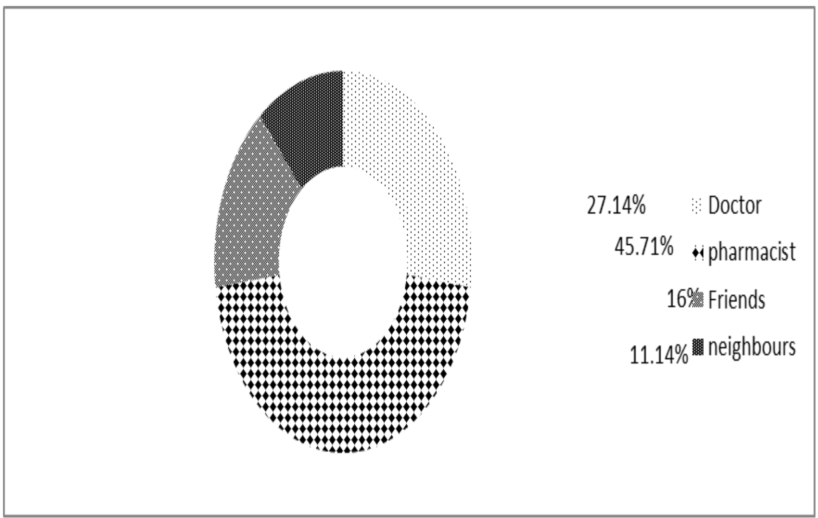

Chart 4: On whom will you Trust while taking OTC drug.

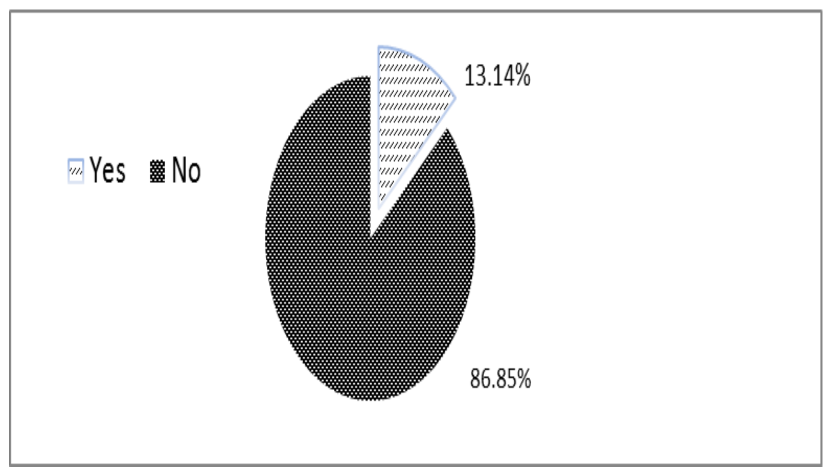

Chart 5: Patient experiencing Side effect of drug.

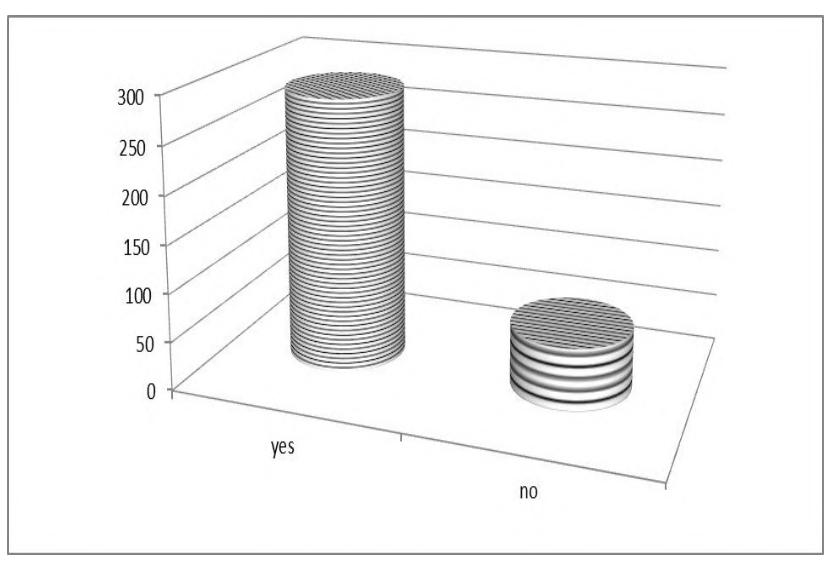

Chart 6: Patient following dose regime.

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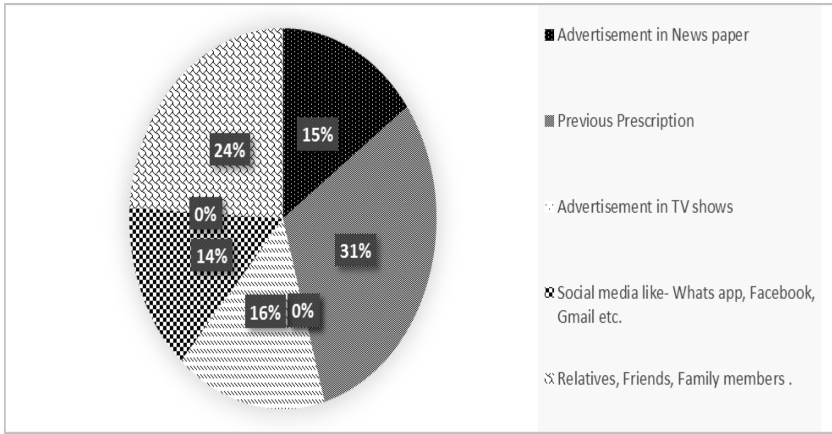

Chart 7: Which information sources used by patient.?

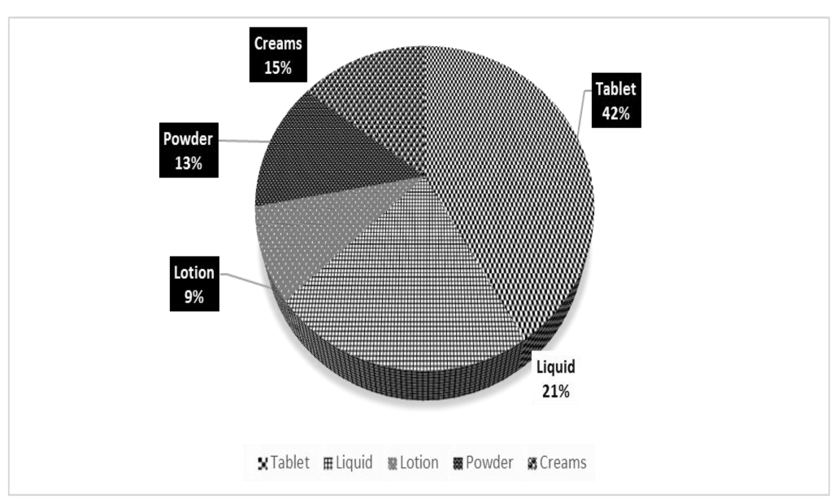

Chart 8: Medicines taken by patient by OTC drug.

RESULT

According to survey in Satara (Wadhe) region it was found that people of age group 50 to 69 experiences acute problems (42.85%) like illness, headache, joint pains and acidity etc. Males took more OTCs (64.28%) than females (35.71%) because from survey it was observed that male has to do hard work, also are having roles and responsibilities, they usually having task to seat in front of PC in industry, so they need to take pain killers and antacid frequently. The patient who took more OTCs was mostly businessman (34.28%) as compared to other occupation because businessman has to manage everything in his business and they are having less time than salaried peoples. Most of people prefer to trust pharmacist (45.71%) while taking OTC drugs rather than doctor, friends and neighbours because it's time saving

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and most often people didn't experience the side effects of drugs(86.85%) but very few experienced side effects (13.14%) such as fever, acidity, skin itching and loss of appetite.

Most of patient followed the dose regime (81.71%) and less percentage of patient didn't follow the dose regime (18.28%) because they didn't remember to take medicine due to their busy schedule. According to survey in Satara (Wadhe) region it was found that amongst several OTC drugs Omeprazole was frequently taken by the patient than any other drugs because most of the people were having problems like acidity, burning sensation in stomach and chest etc.

CONCLUSION

According to survey mostly people above 50-69 of Age. They patient take OTCs and those people OTC as they think that OTC's has less side effect.

Many people follow dose regime while taking OTC drugs. Patient believes on pharmacist for consulting taking OTC's drug from the survey in (Wadhe) Satara region it was conclude that Omeprazole is mostly used OTC Drug.

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SUSTAINED RELEASE MATRIX TYPE DRUG DELIVERY SYSTEM: AN OVERVIEW

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SUSTAINED RELEASE MATRIX TYPE DRUG DELIVERY SYSTEM: AN OVERVIEW

A. Rohit Kumar* and Arjun Sai Sreekar Aeila

GITAM Institute of Pharmacy, GITAM (Deemed To Be University) Rushikonda, Visakhapatnam 530045, Andhra Pradesh, India.

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***Corresponding Author**

A. Rohit Kumar

GITAM Institute of Pharmacy, GITAM (Deemed To Be University) Rushikonda, Visakhapatnam 530045, Andhra Pradesh, India.

ABSTRACT

Sustained drug release formulations are quite helpful in treating chronic diseases. Matrix tablets have been the most likely forms of sustained drug release forms given by oral route. Matrix tablets work by maintaining a constant plasma drug concentration and sustains the rate of release of drug over time and produces therapeutic action for prolonged time period. Extended release plays an important role in formulations having the shorter half-life and high dosing frequency. The matrix controls the rate of release of the drug. Retardants like hydroxy propyl methyl cellulose (HPMC), polyglycolic acid, poly methyl methacrylate are used. The drug is embedded into a matrix core of the retardant. The matrices used may be hydrophobic, bio degradable or mineral types. Different classes of polymers are used in controlling the release of drugs in matrix tablets which may be formulated by wet granulation or direct compression methods. The mechanisms involved in drug release in matrix tablets include both dissolution-controlled and diffusion-controlled. Thus, matrix tablets improve patient compliance by reducing the frequent administration of drug and produce better therapeutic efficacy.

KEYWORDS: Sustained release, Matrix, Polymers.

INTRODUCTION[1,2]

The best route of administration of drugs among all other routes is oral route of Administration. This is because of advantages like low manufacturing cost, ease of administration etc. Many researchers on Rapid and novel delivery had taken place over the past many years. The objective of any drug delivery system is to produce a therapeutic effect

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in the specific site to maintain the aspired drug concentration. For the release of medication for a long period of time After administration of a single dose. Sustained release Matrix tablets are used. Matrix tablets are the best commercial affordable sustained action drugs as they can accommodate large doses of drugs, no special requirements while manufacturing. Sustained release matrix type drug delivery system is the novel drug delivery system (NDDS) which plays an important role in improving the therapeutic effectiveness of the drugs by providing controlled, sustained release and by targeting to the desired site. A constant drug level is maintained for a specific period of time so that the adverse effects are cut down. The basic principle of sustained release drug delivery system is to enhance the pharmacokinetic and pharmacodynamic as well as biopharmaceutical properties in a way where its use is maximized, the side effects are cut down and the disease is cured efficiently when compared to conventional dosage forms.

The dosing frequency is reduced with SRDDS As these optimise pharmacokinetic, biopharmaceutics and pharmacodynamic properties of the drug compared to conventional doses forms. Conventional methods and complex procedures like coating and pelletization is not used in the manufacture.

Advantages[1,2,6]

- Therapeutic concentrations Maintain rate constant levels.
- Drug concentration in blood is uniform.
- Reduction in frequency of administration of dose.
- Ease of manufacturing and cost efficient.
- Accumulation of drug is reduced as the frequency of Administration is less.
- The deficiency in treatment can be improved.
- Compliance problems of the patients are reduced.
- Maximizing bioavailability and minimising local side effects.

Disadvantages[3,4]

- Cost of production is high compared to conventional doses form.
- In vivo and vitro correlation is poor.
- First pass metabolism has increased potential.

Rationale of Developing Sr Material Dds[15,19]

- Reduction in truck frequency.

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- Reduction of toxicity.
- The activity of a drug having less half life is increased.
- Stabilise plasma level drug concentration.

Principle of Sustained Release Drug Delivery Systems[5,8,9]

The active ingredients are released into and absorption pool by the conventional dosage forms. The solution of drug at absorption site is known as absorption pool. Ke,Kr&Ka are first order rate constant of drug elimination, drug release and drug absorption . In conventional doses form drug release immediate showing that Kr>>>>Ka. But Kr <<<< Ka for non immediate release dosage forms, i.e., The rate limiting step is Release of drug doses form. Zero order kinetics is seen and that is shown by the equation.

$$Kr^{\circ} = \text{Rate In} = \text{Rate Out} = KeCd\ Vd$$

Where, K: Zero-order rate constant for drug release- r°Amount/time, K : First-order rate constant for overall e drug elimination-time, C: Desired drug level in the d body – Amount/volume, and V: Volume space in d 4 which the drug is distributed in litre.

Factors Considered In Dosage Form Design[7,11,12]

There are mainly 2 kinds of factors that effect the dosage form design. They are divided into:

1. **Biological factors**

a. First pass effect: Drugs which suffer an extensive first pass effect shows retarded release rate. This retarded release rate affects the bioavailability.

b. Half life: The half-life of a drug is the measure of its time of residence in the body. If the medication has a short half-life (less than 2 hours), a prohibitively large amount of the drug may be found in the dosage form. On the other hand, a drug with a half-life of removal of eight hours or more is adequately maintained in the body when administered in traditional doses and continuous delivery of drugs systems

c. Adverse effects: Prolonging the drug release may develop undesirable adverse reactions.

d. Absorption and solubility: absorption and solubility both are interlinked. incorporation of drugs which are poorly water soluble can cause the reduction in overall absorption efficiency.

2. Physiochemical Factors

a. **Drug stability:** The important factor in oral dosage forms is the loss of medication in the GI tract by means of acid hydrolysis and/or metabolism. While a drug undergoes degradation in solid states at a much slower rate than a suspended or solution substance. It

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is possible to significantly improve the relative bioavailability of a medication that is toxic in the stomach; the most effective control unit would be one that activates its substance only in the intestine.

b. **Aqueous solubility & Pka:** A medication to be absorbed and dissolved in the aqueous phase adjacent to the route of administration site and then partitioned into the absorbing membrane. Two of the most important physicochemical properties of a drug that affect its absorption activities are its aqueous solubility and, if it is soft acid, its pKa. Such properties reward a dominant role in the success of controlled release schemes. Drugs with high aqueous solubility have poor degradation levels and are typically susceptible to oral bioavailability tribulations.

c. **Partition Coefficient:** It is the ratio of the drug in the oil phase to that of the aqueous phase. Drugs having higher partition co efficient are not suitable for oral SRDDS as they won't partition out of the lipid membrane once it gets in the membrane . It can be calculated by the formula

$$K = Co / Cw$$

Co = Equilibrium concentration in organic phase

Cw= Equilibrium concentration in aqueous phase

d. **Diffusivity and molecular size:** The membrane cavitie's size and shape influences the diffusivity. Ntermediate molecular weight drug diffusion coefficient is 100-400 Daltons; 10-6-10-9 cm2/sec is due to flexible polymer array. For drugs having molecular weight > 500 Daltons, the diffusion coefficient in many polymers are very less i.e. less than 10-12 cm2/sec. Proteins and peptides are examples of drugs that are difficult to control drug release level from dosage form.

Requirements To Be Met To Incorporate Drug Into Sustain Release Dosage Form[17,18]

Both the physiochemical and pharmacokinetic properties had to be considered while incorprating the drug into SRDDS:

Table No 1: Physicochemical Parameters for Drug Selection.

Parameters	Criteria
Molecular size	< 1000 Daltons
Aqueous Solubility Apparent partition coefficient	More than 0.1 mg/ml for pH 1 to pH 7.8 High
Absorption mechanism	Diffusion
General absorbability From all GI segments	Release Should not be influenced by pH and enzymes

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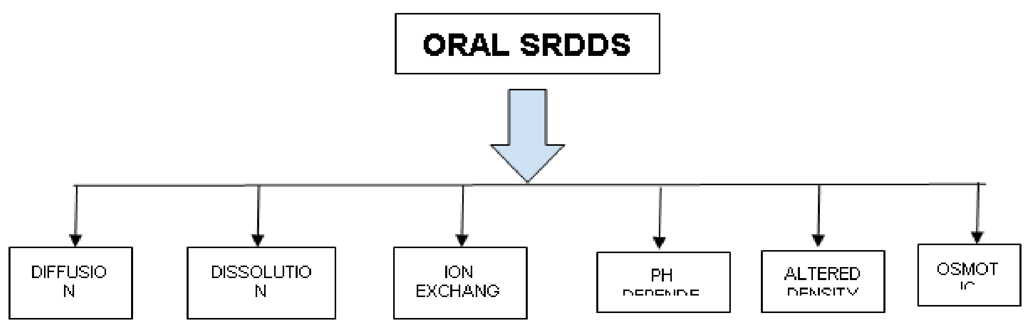
Table No.2: Pharmacokinetic Parameters for Drug Selection.

Parameters	Comment
Elimination half-life	Between 2 to 8 hrs
Absolute bioavaliability	Should be 75% or more
Absorption rate constant (Ka)	Must be higher than release rate
Apparent volume of distribution(Vd) Required for design	Larger Vd and MEC, Larger will be the required dose
Total clearance	Not depend on dose
Elimination rate constant	Required for design
Therapeutic concentration (Css)	The lower Css and smaller Vd, the loss among of drug required.
Toxic concentration	Apart the value of MTC And MEC safer the dosage form

Formulation Strategies[13,14,16]



1. **Dissolution sustained systems:** A product that naturally retains this drug at a slow dissolution rate and reduces its dissolution rate by sufficient salt or derivative formation for those drugs with high water solubility. Generally speaking, these devices are used in the processing of enteric coated dosage forms.Stomach safety from the effects of drugs like Aspirin is used, a coating that dissolves in natural or alkaline water. It delays drug release from the dosage process until the lower pH of the intestine is achieved.
2. **Diffusion sustained system:** It involves the passage of drug molecules from higher concentration to the lower concentration. The flux of drug is given by

   J= - D dc/dx

   D = diffusion coefficient in area/ time dc/dx = change of concentration 'c' with distance ‘x’
3. **pH– Independent formulations:** Maintain the constant pH , help to make pH-independent drug release substitutes such as amino acid salts, citric acid, phthalic acid, phosphoric acid and tartaric acid applied to the formulation. Preparation of buffered sustained release formulation is generally done by combining a simple or acidic product with one or more buffering agents, granulating with suitable pharmaceutical excipients, and covering with permeable film forming polymer with gastrointestinal fluid. As

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gastrointestinal fluid permeates through the membrane, the buffering agents change the fluid inside by making a constant drug release rate to the correct constant pH.

4. **Ion exchange:** Using ion exchange resin is an appealing strategy for continuous drug delivery as the characteristic of drug release depends largely only on the ionic environment of drug-containing resins and is less sensitive to environmental conditions such as enzyme content and pH at the absorption site zero order release kinetic can be accomplished satisfactorily using this approach.
5. **Altered density:** Not releasing of all the drug contents in GIT causes a limited use, to over come this various methods are developed to increase the resident time in GIT.

a. **High density Approach:** The density of the pellets should be 1-4 gm/cm3 which is more than that of the stomach contents. The drug is coated with heavy inert materials like Zinc Oxide.

b. **Low density approach:** lobular shells with a thickness smaller than that of gastric fluid used as a product carrier for sustained release purposes such as polystyrene, pop rice and popcorn are all used as carriers to undercoat the surface of these empty shells with sugar or polymeric materials such as methacrylic polymer and cellulose acetate phthalate. A mixture of product with polymer such as ethyl cellulose and hydroxy propyl cellulose then coats the undercoated shell. The final product thus remains on the gastric fluid for a long time, while the substance is gradually released.

Table No.3: Examples of Polymers.

TYPE	EXAMPLE
Soluble polymers	Polyethylene glycol (PEG) Polyvinyl alcohol (PVA)
Biodegradable polymers	Polyacetic acid (PLA) Polyglycolic acid (PGA) Polyanhydrates
Hydrogels	Poly-hydroxyethyl methacrylate (PHEMA) Cross-linked polyvinyl alcohol (PVA) Polyacrylamide(PA)
Mucoadhesive polymers	Polycarbophil Sodium carboxymethylcellulose Tragacanth Methylcellulose
Non-biodegradable polymers	Cellulose acetate (CA) Polyethylene vinyl acetate (PVA) Polyether urethane (PEU)
Natural gums	Xanthan gum Guar gum Karaya gum Gum arabic

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Classification of Matrix Tablets[10,17]

Matrix tablets can be classified as;

A) On the basis of retardant materials used :

Under this category the matrix tablets are further divided into 5 types:

a) Hydrophobic matrices (plastic matrices)
b) Lipid matrices
c) Hydrophilic matrices
d) Bio-degradable matrices
e) Mineral matrices.

B) On the basis of porosity of matrix:

a) Macroporous systems
b) Microporous systems
c) Non-porous systems.

A) ON THE BASIS OF RETARDANT MATERIAL USED

a) Plastic matrices or hydrophobic matrices

Plastic matrices were first introduced in 1959 by using hydrophobic/ inert materials. In this method; firstly, the drug was mixed with a hydrophobic polymer and then compressed into a tablet. The dispersion of the drug is achieved by the diffusion across a network of channels which exists between compact powder particles. Thus, sustained release is produced. The hydrophobic matrices are made by using polyethene, poly-vinyl chloride and acrylate polymers and their co-polymers. These matrix tablets are inert in nature due to the presence of water and gastro- intestinal fluids. The mechanism of these matrix tablets is diffusion and the liquid penetration is the rate limiting step.

b) Lipid matrices

Lipid waxes are used in the preparation of these matrices. The drug is released from these matrices through pore diffusion and erosion. The sustained release through these matrices is more sensitive to digestive fluid composition when compared to totally insoluble polymer matrix. Carnauba wax is combined with stearyl alcohol/ stearic acid to form the retardant base for most of the sustained release formulation.

c) Hydrophillic matrices

A matrix is defined as a properly mixed composite of one or more drugs using a hydrophilic polymer (gelling agent). The hydrophilic polymer matrix is often used in oral controlled drug

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delivery because of the efficiency in obtaining a desirable drug release profile, cost effectiveness and broad regulatory acceptance. These matrices are further divided into three groups based on the polymers used;

- **Cellulose derivatives**

The polymers used in the formulation are methylcellulose 400 and 4000cps, hydroxypropylmethyl cellulose (HPMC) 25, 100, 4000 and 15000cps, hydroxyl ethyl cellulose and sodium carboxymethyl cellulose.

- ***Non-cellulose natural/semi—synthetic polymers***

Polymers of acrylic acid: The most widely used polymer under this category is carbopol-934. Other polymers include agar-agar, alginates, carob gum, molasses, polysacharrides of galactose and mannose, chitosan and modified starches.

d) Biodegradable Matrices

Polymers linked to one another via functional groups having unstable linkage in the backbone, are present in biodegradable matrices. These matrices are degraded biologically and the enzymes which are generated by the surrounding living cell erode the matrix. The degradation can occur by non enzymatic process by metabolising the oligomers and monomers. Natural polymers include proteins and polysaccharides. It also contains certain modified natural polymers. The synthetic polymers include aliphatic polyesters and poly anhydrides.

e) Mineral matrices

Mineral matrices contain polymers obtained from different species of seaweeds. Alginic acid, a hydrophilic carbohydrate obtained from species of brown seaweeds by using dilute alkali, is an example of mineral matrices.

B) ON THE BASIS OF POROSITY OF MATRIX

In this the drug molecules diffuse across the matrix and produce sustained release.

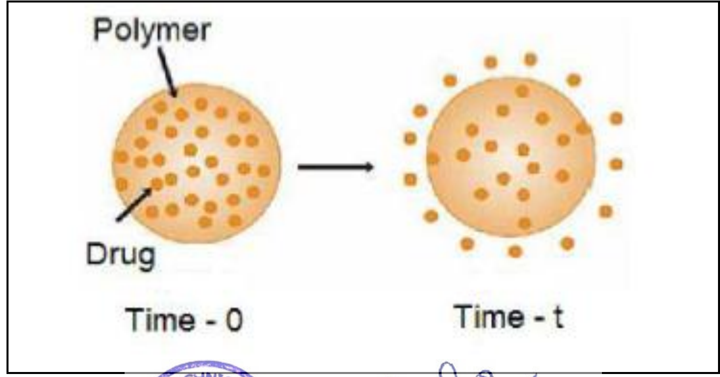

Figure: Diffusion of Drug Across The Matrx.

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The matrix is further divided into 3 types.

a) Macro porous systems

The pores of this kind of matrix range from 0.1µm to 1µm which is larger than the diffusant molecule size. In this type of system permeation of drug occurs through these pores.

b) Micro porous systems

Permeation of drug molecules occurs through pores of sizes ranging from 50-200Å.

c) Non-porous systems

These systems have no pores. The diffusion of molecules occurs through network meshes. There is no pore phase where as the polymeric phase is present.

CONCLUSION

The review of the above article is mainly focused on the formulation and uses of the SRDDS. It concludes that the use of matrix tablets was really helpfull to over come the patient complience problems which are associated with the conventional dosage forms . The cost of production of the matrix tablets is also under control. Due the use of these tablets the daily required frequence of the doses was also reduced.

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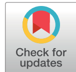
Citric acid crosslinked carboxymethylcellulose-polyvinyl alcohol hydrogel films for extended release of water soluble basic drugs



Vishwajeet Sampatrao Ghorpade[a,*], Remeth Jacky Dias[b], Kailas Krishnat Mali[a], Safina Ismail Mulla[a]

[a] Department of Pharmaceutics, YSPM's Yashoda Technical Campus, Wadhephata, Satara, 415011, Maharashtra, India
[b] Department of Pharmacy, Government Polytechnic, Jalgaon, Maharashtra, India

ARTICLE INFO

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ABSTRACT

The aim of present work was to develop carboxymethylcellulose (CMC)-polyvinyl alcohol (PVA) hydrogel films for extended delivery of water soluble basic drug, using citric acid (CA) as a cheap and non-toxic crosslinking agent. Gentamicin sulfate (GTM) was used as a model drug. The hydrogel films were evaluated for carboxyl content, tensile strength, swellability, drug loading and release, hemocompatibility and characterized by ^{13}C-CP-MAS NMR, ATR-FTIR and thermal analysis (TGA and DSC). The instrumental analysis helped to confirm the formation of ester crosslinks. CMC-PVA hydrogel films exhibited greater carboxyl content, tensile strength and swellability than the pure CMC hydrogel films. The GTM loading increased with an increase in the amount of PVA in the hydrogel films. The CMC-PVA hydrogel films showed propensity to extend the release of GTM above 24 h. Hemolysis assay revealed the hemocompatible nature of the hydrogel films. Altogether, the CMC-PVA hydrogel films can be envisioned as promising biomaterial for the delivery of water soluble basic drugs.

1. Introduction

Hydrogels are the crosslinked polymeric network structures capable of absorbing large amount of water without dissolving in it. Due to their rubbery consistency and water retention capacity which resembles natural tissues, hydrogels are mostly used in the field of biomedical and pharmaceutical sciences [1]. The hydrogels can be prepared from synthetic or natural polymers using physical, chemical or radiation crosslinking. The hydrogels prepared using synthetic polymers exhibit good mechanical properties; however, most of them do not support cell adhesion and tissue formation. On the other side, natural polymer based hydrogels are biocompatible, biodegradable and support cellular activities [2]. In recent years, extensive research has been carried out to develop hydrogel-based drug delivery systems using natural polymers [3–9]. Amongst the natural and semi-synthetic polymers, cellulose and its water soluble derivatives are mostly preferred because of the abundance of cellulose on earth and relatively low cost of the cellulose derivatives than the synthetic polymers [10].

Carboxymethylcellulose (CMC) is highly hydrophilic cellulose derivate [11,12]. It is widely used as thickening and suspending agent in the food and pharmaceutical industry. The scientists dealing with the hydrogels for drug delivery are interested in CMC due to its good swellability, non-toxicity and modifiability [13]. Large number of reports are available on the preparation of CMC-based hydrogels for the delivery of water soluble drugs [14–17]. In most of these cases, the crosslinking agents used are either expensive or toxic [18]. In last few years, citric acid (CA) has emerged as a non-toxic and cheap crosslinking agent. At high temperatures, it esterifies the hydroxyl groups present on the nearby polymer chains and forms the crosslink. For preparation of CA crosslinked hydrogels of alone CMC, excess of CA is required as CMC shows poor crosslinking due to electrostatic repulsion in between the carboxylate ions present on the adjacent polymer chains. This leads to the formation of intramolecular crosslinks. Therefore, Demitri and co-workers (2008) fabricated CA crosslinked superabsorbent hydrogels by combining CMC with hydroxyethylcellulose (HEC) for improving the intermolecular crosslinking [19]. These hydrogels were intended for agricultural application. The hydroxyl groups attached to the oxyethylene chains in HEC are highly reactive and hence, can readily undergo esterification reaction with CA and facilitate the formation of CMC hydrogels with good integrity. Very few works are reported on the use of CA crosslinked CMC based hydrogels in drug delivery. Mali et al. (2018) have prepared hydrogel films comprised of CMC and tamarind gum (TG) for delivery of moxifloxacin HCl. Furthermore, we have prepared CMC-polyethylene glycol

* Corresponding author.
E-mail address: vsg.bpharm@yes.edu.in (V.S. Ghorpade).

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(PEG) hydrogel films for the improvement of loading and extended release of hydrophobic drugs [20].

Polyvinylalcohol (PVA) is a synthetic polymer which is biocompatible, biodegradable and widely used in pharmaceutical, bio-medical and food sectors [21]. The large number of reactive hydroxyl groups present on the polymer chain of PVA makes it suitable for the synthesis of hydrogels, mostly by physical [22–24] or chemical [25–28] crosslinking. The CA crosslinked PVA films have been prepared for antimicrobial packaging purpose [29]. Taking this into consideration, PVA can be used as a better alternative to HEC while preparing CMC hydrogels since the amount of PVA required for the formation of intact hydrogels will be less. In previous studies, CMC-PVA hydrogels have been prepared for drug delivery [30–32]; however, CA crosslinked CMC-PVA hydrogels have not been investigated for their ability to control the drug release.

The present work deals with the preparation of CMC-PVA hydrogel films using CA as crosslinking agent, for the delivery of water soluble basic drugs. Our objective was to develop the inexpensive hydrogels with good drug loading efficiency and ability to control the release of water soluble drugs, so that they can be used as implants or wound dressings. As the crosslinks consist of COOH branching which usually ionize at physiological pH, the prepared hydrogel films could be more favorable for the delivery of basic drugs. Along with an improvement in the extent of crosslinking, the large number of OH groups on PVA chains may also contribute towards the enhancement of swellability of the CMC-PVA hydrogel films. Also, the OH groups of PVA and COOH groups of CA crosslinks may hold the weakly basic drug molecules (pKa > physiological pH) within the hydrogel matrix by hydrogen bonding and electrostatic interactions. Taking these aspects into account, we hypothesize that the CA crosslinked CMC-PVA hydrogel films may enhance the loading as well as control the release of water soluble basic drug.

The hydrogel films were suitably characterized to confirm the formation of ester crosslinks. The films were loaded with gentamicin sulfate (GTM, model water soluble basic drug) and evaluated for drug release. Hemolysis assay was performed to test the hemocompatibility of the hydrogel films.

2. Materials and methods

2.1. Materials

Gentamicin sulfate (GTM) was obtained as a gift sample from RP Pharma, Mumbai, Maharashtra (India), sodium carboxymethylcellulose (CMC, degree of substitution: 0.7, average molecular weight: ~250000) was purchased from Sigma Aldrich, Mumbai, Maharashtra (India), polyvinyl alcohol (PVA, MW: 115,000 Da, Hydrolysis: 98–99%) and citric acid (CA) were purchased from Loba Chemie, Mumbai, Maharashtra (India). All other chemicals were of analytical grade and used as received.

2.2. Preparation of CMC-PVA hydrogel films

The CMC-PVA hydrogel films were prepared by modifying the previously reported method [20]. Initially, a suitable amount of PVA was dissolved in distilled water by heating on a water bath at 80 °C for 20 min, under reflux. The obtained PVA solution was cooled to room temperature followed by addition of CMC and CA (% of polymer amount). This mixture was stirred on a magnetic stirrer to obtain a homogenous solution and placed overnight in a closed compartment in order to remove the air bubbles. The clear solution was poured into the petri dish of specific diameter (9 cm) and dried in the hot air oven at 50 °C for 24 h. The dried CMC-PVA-CA film was cured at 145 °C for 5 min, to promote the crosslinking reaction in between the polymer chains. The cured film was washed with distilled water in cycles until the pH of water became nearly neutral. Finally, the swollen film was washed with isopropyl alcohol to remove the water entrapped within the matrix. The shrunken film so obtained was dried in the hot air oven at 40 °C for 24 h and stored in a desiccator. The concentration of PVA was varied in order to study their effect on the film properties (see Table 1).

Table 1
CMC-PVA hydrogel films containing different concentrations of PVA.

Batch	CMC (%w/v)	PVA (%w/v)	CA (%)[a]
HF0	2	0	20
HF0.5	2	0.1	20
HF1	2	0.2	20
HF2	2	0.4	20
HF3	2	0.6	20

[a] Indicates % of the polymer amount.

2.3. Determination of carboxyl content

The total carboxyl content of hydrogel films was determined by acid-base titration [33,34]. CO_2 free water was used for the preparation of titrant (0.1 N HCl) and analyte (0.1 N NaOH). A specific amount of hydrogel film was added to the adequate volume of 0.1 N NaOH and stirred on a magnetic stirrer for 2 h. The hydrogel films disintegrated and dissolved completely due to sodium hydroxide aided breaking of the ester crosslinks. Also, sodium hydroxide reacts with the free carboxylic acid groups to form sodium citrate. The excess amount of 0.1 N NaOH was titrated with 0.1 N HC1 using phenolphthalein indicator. The carboxyl content in milliequivalents per 100 g of hydrogel film was determined using following formula:

$$Total\ carboxyl\ content\ (mEq/100\text{g}) = \frac{(V_b - V_a) \times N_{HCl} \times 100}{W_{HF}} \quad (1)$$

where, N_{HCl} is the normality of HCl (mEq/mL), V_b and V_a are the volumes of HCl in absence and presence of hydrogel film, and W_{HF} is the weight of hydrogel sample (g).

2.4. Characterization of hydrogel films

Solid state ^{13}C cross-polarization-magic angle spinning (^{13}C CP-MAS) NMR spectra of CMC, PVA, CA and CMC-PVA hydrogel film (HF3) was recorded using JEOL-ECX400 spectrometer operated at 400 MHz, with a spinning speed of 10 KHz. The contact time, relaxation delay and sweep width were fixed at 3.5 ms, 5 s and 35 kHz, respectively. The external hexamethylbenzene standard methyl resonance at 17.3 ppm was used for the calibration of chemical shifts.

The infrared spectra of CMC, PVA, CA and hydrogel films were obtained using ATR-FTIR spectrophotometer (MIRacle-10, IR Affinity-1, Shimadzu, Japan). The samples were transferred to the ATR compartment and spectra were obtained in the range of 400–4000 cm^{-1} (scans = 25; resolution = 4 cm^{-1}).

CMC, PVA and hydrogel films (HF0 and HF3) were subjected to the thermal analysis (TGA and DSC) using SDT Q600 V20.9 Build 20 (TA instruments, Water, USA). Samples (approx. 5 mg) were taken in the sample pan and were heated from 30°C to 300 °C, at the heating rate of 10 °C/min, under nitrogen atmosphere (purge rate = 10 mL/min).

2.5. Determination of tensile strength

The tensile strength of the hydrogel films was tested using Texture Analyzer (CT 3-10,000, Brookfield, WI) [35,36]. The analyzer was fitted with a 10 kg load cell. Each film was cut into 2 cm × 1 cm size and clamped on probe TA-DGA (hold time = 60 s). Holding the lower clamp stationary, the hydrogel film was pulled apart with the help of upper clamp with a speed of 2 mm/s to a distance of 6 mm (trigger load = 0.05 N). The force at which the film broke was recorded and the

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tensile strength of the hydrogel films was determined as given below.

$$Tensile\ strength\ (kg/mm^2) = \frac{Force\ at\ break}{Initial\ cross - sectional\ area} \quad (2)$$

2.6. Determination of swelling behavior

The swelling behavior of the hydrogel films was studied in phosphate buffer (pH 7.4) at 37 °C. The hydrogel films (~0.2 g) were immersed in the beaker containing 20 mL buffer. After 30 min, swollen film samples were removed from the buffer and their surface was blotted with tissue paper to remove the excess water followed by weighing on the analytical balance (Shimadzu, AX 120). The hydrogel films were reimmersed in the buffer and the procedure was repeated at specific time intervals till 24 h. The swelling ratio of the hydrogel films was determined by using eq. no. (3).

$$Swelling\ ratio\ (g/g) = \frac{W_s - W_d}{W_d} \quad (3)$$

where, W_s is the weight of swollen hydrogel film and W_d is the weight of dry hydrogel film. The measurements were taken in triplicate [3,37].

2.7. GTM loading

In order to load GTM into the hydrogel films, the preweighed films (~100 mg) were allowed to swell in the aqueous GTM solution (10 mg/mL) till equilibrium. The swollen hydrogel films were dried at room temperature for 24 h. In order to determine the amount of GTM loaded in the hydrogel films, they were initially cut into fine pieces. The specific amount of these pieces were added to 50 mL phosphate buffer (pH 7.4) and stirred for 48 h. Thereafter, the amount of GTM released in the buffer was determined using colorimetric estimation as GTM shows negligible UV absorbance [38]. Briefly, standard solutions of GTM (5–9 μg/mL) were prepared in phosphate buffer (pH: 7.4). One mL of these solutions was taken in the 10 mL volumetric flasks followed by addition of 0.5 mL of 2 M H_2SO_4 and 2 mL of 5×10^{-4} M $KMnO_4$ solutions. The obtained mixtures in the flasks were heated on the water bath for 25 min, cooled to room temperature and 2.5 mL of 5×10^{-4} M methylene blue was added to the respective flasks. The volume was adjusted to 10 mL with buffer and the mixtures were subjected to spectrophotometric analysis (UV1800, Shimadzu, Japan) at 664 nm. The calibration curve so obtained was used further for determination of GTM released in the buffer [39].

2.8. In vitro GTM release

The in vitro drug release study was performed by modifying the previously reported methods [40,41]. The GTM loaded hydrogel films of known weight were placed in screw-capped vials containing 10 mL of phosphate buffer (pH: 7.4). These vials were kept in the constant temperature bath (without agitation) at 37 °C. At specific time intervals, aliquots were withdrawn and suitably diluted followed by spectrophotometric analysis (UV1800, Shimadzu, Japan) using colorimetric method as described above. All the measurements were taken in triplicate.

2.9. Hemocompatibility study

Recently, biocompatibility of natural polymer based biomaterials was tested by few authors using hemolysis assay [5,7,9]. As the CMC-PVA hydrogel films can be used as implants or wound dressings, they were examined for hemocompatibility using hemolysis assay with slight modification [42]. The hydrogel films were cut in order to get surface area of 2 cm^2. These films were allowed to swell in the phosphate buffer saline (PBS) maintained at 37 °C for 1 h. Thereafter, PBS was removed and 0.5 mL of human CPD (citrate-phosphate-dextrose) blood was added over the films. After 20 min, the hemolysis process was stopped by addition of 4 mL, 0.9% NaCl saline followed by incubation of the samples for 1 h at 37 °C. The incubated samples were centrifuged at 4000 rpm for 10 min and the supernatant was subjected to spectrophotometric analysis (UV1800, Shimadzu, Japan) at 545 nm. The hemolysis (%) was determined using following formula:

$$Hemolysis\ (\%) = \left(\frac{A_{Test\ sample} - A_{-ve\ control}}{A_{+ve\ control} - A_{-ve\ control}}\right) \times 100 \quad (5)$$

where, A = absorbance.

+ve control = mixture of 0.5 mL, human CPD blood and 4 mL double distilled water
- ve control = mixture of 0.5 mL, 0.9% NaCl saline and 4 mL double distilled water

2.10. Statistical analysis

The experimental data were compared by one-way analysis of variance (ANOVA) using GraphPad Prism 5.01 software. The statistical significance was considered at $p < 0.05$.

3. Results and discussion

3.1. Formation of CMC-PVA hydrogel films

The CMC-PVA hydrogel films were obtained by esterification-crosslinking mechanism. At high temperatures, CA gets converted into an anhydride which esterifies the OH groups of CMC and PVA. This leads to the formation of ester crosslinks in between the polymer chains. Fig. 1 displays the possible reaction involved in the formation of CMC-PVA hydrogel films. Besides CMC-PVA crosslinks, there may be presence of CMC-CMC and PVA-PVA crosslinks in the hydrogel matrix. The concentration of CA, curing temperature and curing time required for the formation of the hydrogel films was decided on the basis of preliminary studies. The minimum concentration of CA needed for the formation of hydrogel film with good matrix integrity was 20%. On increasing the CA concentration above 20%, the hydrogels exhibited poor swellability. Although the previous reports indicate the formation of hydroxyl acid crosslinked cellulose-based hydrogels at the curing temperature of 80 °C, the time required for the curing was more (> 10 h) [19,43]. In the preliminary studies, we found that the curing temperature of 145 °C and curing time of 5 min was sufficient to obtain the hydrogel films with desired swellability and integrity. However, increasing the curing temperature and curing time above 145 °C and 5 min led to the development of yellowish color on the films, possibly due to thermal decomposition. The obtained hydrogel films showed an average thickness in the range of ~130–135 μm.

3.2. Total carboxyl content

Table 2 represents the total carboxyl content of the CMC-PVA hydrogel films. It was observed that an increase in the amount of PVA up to 0.2% increased the carboxyl content of the hydrogel films. In case of CMC hydrogel film (HF0), the carboxyl content was found to be less as compared to the CMC-PVA hydrogel films. This indicates that CMC hydrogel film showed minimum number of interpolymer crosslinks than the CMC-PVA hydrogel films. This can be attributed to the degree of substitution (DS) of CMC i.e. 0.7 and weak electrostatic repulsion in between the adjacent CMC chains due to presence of few COO^- groups in the feed of HF0. The DS equivalent to 0.7 suggests that very few primary hydroxyl groups (C6-OH) are available on CMC for taking part in the esterification reaction. On the other hand, the weak electrostatic repulsion in between the adjacent CMC chains may inhibit the CA from linking them. As a result, most of the CA may remain unutilized. PVA

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Fig. 1. Reaction involved in the formation of CMC-PVA hydrogel films.

consists of large number of secondary hydroxyl groups which are highly reactive [44]. These hydroxyl groups can readily participate in the esterification reaction. On inclusion of PVA in the feed, the CA which was unutilized in case of CMC hydrogels will get involved in the formation of CMC-PVA and PVA-PVA crosslinks. This may be the reason behind increase in the carboxyl content of HF0.5 and HF1. However, further increase in the amount of PVA (HF2 and HF3) led to decrease in the carboxyl content possibly due to decrease in the crosslinking density of the hydrogel films caused by dilution effect. In case of batch HF1 (0.2% PVA), there might be a possibility of maximum consumption of the citric acid in the formation of crosslinks. As a result, further increase in the concentration of PVA may increase the distance in between the adjacent crosslinks within the hydrogel films and reduce the crosslinking density. Another reason behind decrease in the crosslinking density on increasing the concentration of PVA beyond 0.2% may be insufficient physical interactions in between CMC and PVA at high concentrations of PVA. HF3 containing high amount of PVA showed low carboxyl content than HF1 and HF2 but it was greater than HF0.

Table 2
Total carboxyl content (TCC), tensile strength (TS), equilibrium swelling ratio (ESR), GTM loading and hemolytic activity of the CMC-PVA hydrogel films.

Batch	TCC (mEq/100 g)	TS (kg/mm^2)	ESR (g/g)	GTM loading[a]	Hemolysis (%)
HF0	229.55	3.13 ± 0.16	50.51 ± 3.63	137.51 ± 1.56	4.15 ± 0.22
HF0.5	391.20	3.38 ± 0.09	53.55 ± 2.75	162.24 ± 2.12	4.52 ± 0.83
HF1	417.35	3.54 ± 0.12	59.75 ± 1.90	190.40 ± 1.04	4.02 ± 0.71
HF2	372.81	3.49 ± 0.05	66.42 ± 1.88	204.22 ± 2.13	3.87 ± 0.54
HF3	354.91	3.45 ± 0.13	77.31 ± 2.96	216.03 ± 1.89	4.34 ± 0.48

[a] Indicates readings in mg/g of hydrogel films.

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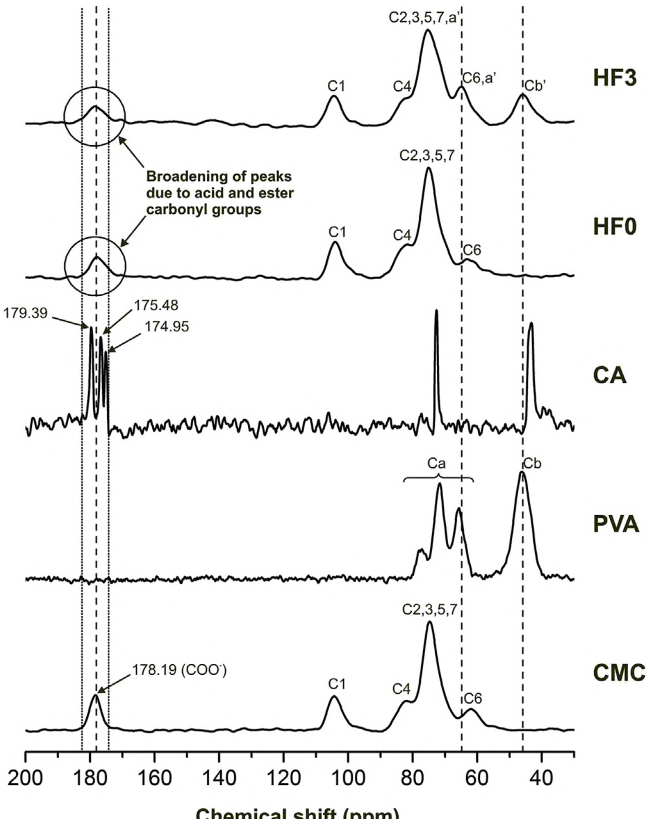

Fig. 2. Solid state ^{13}C-NMR of CMC, PVA, CA, HF0 and HF3.

3.3. Solid state ^{13}C-NMR analysis

The solid state ^{13}C-NMR spectrum of CMC, PVA, CA, CMC hydrogel film (HF0) and CMC-PVA hydrogel film (HF3) is shown in Fig. 2. The symbolic representation of various carbon atoms of CMC and PVA is indicated in Fig. 1. The carbon atoms of CMC showed a signals in the region 56-66(C6), 68-80(C2,3,5,7), 81-89(C4), 98-109(C1) and 175-183(COO^-) ppm. The resonance peaks corresponding to the carbon atoms of PVA appeared at 46.16 ppm (Cb) and in the range of 60–80 ppm (Ca). The carbonyl carbons of citric acid showed sharp signals at 174.95, 175.48 and 179.39 ppm. The spectrum of CMC-PVA hydrogel film (HF3) showed existence of new peaks at 45.90 and 64.70 ppm which represent the peaks of PVA (Cb at 46.16 ppm and Ca at 65.64 ppm). These peaks were absent in the spectrum of pure CMC and CMC hydrogel film (HF0). The remaining peaks of PVA were not visible in the spectrum of HF3 because they overlapped with the peaks of carbons belonging to the anhydroglucose units of CMC. Besides, the sharp carboxylate carbon peak of CMC at 178.19 ppm was found to be slightly broadened towards up-field region in the spectrum of HF0 and HF3. This clearly indicates the presence of ester crosslinks in the hydrogel films.

3.4. ATR-FTIR analysis

The presence of ester crosslinks in the CMC-PVA hydrogel films was further confirmed from ATR-FTIR analysis. Fig. 3a depicts the ATR-FTIR spectra of CMC, PVA and CA, whereas spectra of hydrogel films (HF0-HF3) are presented in Fig. 3b. The spectrum of CMC showed the characteristics peaks at 3450 cm^{-1} (O-H stretching), 2920.52 cm^{-1} (C-H stretching), 1584 cm^{-1} (C=O stretching) and 1051 cm^{-1} (C-O stretching). In the spectrum of PVA, broad band was observed at 3257 cm^{-1} corresponding to O-H stretching and sharp peaks at 2929 cm^{-1} represented C-H stretching. The peaks of PVA at 1427 cm^{-1}, 1327 cm^{-1} and 839 cm^{-1} were related to the C-H bending whereas the prominent peak at 1082 cm^{-1} represented C-O stretching. The distinct IR peaks of CA were observed at 3498 cm^{-1} and 3283 cm^{-1} (O-H stretching), 1693 cm^{-1} (C=O stretching), 1199 cm^{-1} (C-O stretching) and 786 cm^{-1} (C-C stretching).

The spectrum of HF0 showed peak at 1722 cm^{-1} corresponding to the ester as well as carboxylic acid carbonyl stretching. This indicates the presence of ester crosslinks in the CMC hydrogel film (see Fig. 3b). The changes in the carbonyl peak of the hydrogel films on addition of PVA can be clearly observed in Fig. 3c. The intensity of this peak was found to be increased as we move from HF0 to HF1, which suggests an increase in the carboxyl content and hence, the extent of crosslinking of the hydrogel films as the concentration of PVA is increased up to 0.2%. As the concentration of PVA was further increased (HF2 and HF3), the carbonyl peak intensity was found to be reduced. This clearly reveals that increase in the concentration of PVA reduces the crosslinking density. The results of carboxyl content determination were in accordance with this finding. It was also noticed that the carbonyl peak shifted towards lower wavenumber with increase in the amount of PVA from 0.2% to 0.6% (HF1-HF3). An increase in the hydrogen bonding interaction in between the OH groups of PVA and carbonyl groups of free acid and ester groups of the CMC-PVA hydrogel films may be responsible for this shifting. The intensity of peak at 2942 cm^{-1} was found to be increased from HF0 to HF3 (see Fig. 3b) which can be attributed to an increase in the number of CH_2 groups associated with PVA.

3.5. Thermal analysis

Fig. 4 depicts the TGA, DTG and DSC profiles of CMC, PVA, HF0 and HF3. The TGA thermogram of CMC showed two stages whereas that of PVA showed three stages of mass loss (see Fig. 4a). In case of CMC, 10.56% mass loss occurred in between 40 °C to 163 °C due to loss of moisture followed by 42.19% mass loss in the range of 253 °C–316 °C, owing to thermal decomposition of the sample. The maximum degradation temperature (T_m) of CMC was observed at 295.11 °C (see Fig. 4b). For PVA, the initial mass loss due to moisture evaporation took place within the temperature range of 60–190 °C. The major loss (61.96%) occurred in between 239 °C to 374 °C which can be ascribed to the elimination of hydroxyl groups in the form of water and formation of polyene macromolecules [45]. The T_m of PVA was noticed at 305.06 °C. In the third step (410–476 °C), decomposition of macromolecules led to the mass loss of 10.98%. The batch HF0 exhibited degradation in two stages (I: 40–174 °C; II: 223–350 °C) with total weight loss of 62.3% at 485 °C whereas HF3 showed three stages of degradation (I: 30–176 °C; II: 234–343 °C; III: 418–480 °C) to due to presence of PVA. The total weight loss for HF3 at 485 °C was found to be 67.24% which was slightly greater than HF0. Also, T_m of HF3 was found to be reduced when compared with HF0. This clearly reveals that the crosslinking density of HF3 was less than HF0.

The values of the glass transition temperature (T_g) obtained from the DSC curves of HF0 and HF3 helped to reconfirm our above statement (see Fig. 4c). The T_g of HF3 was lower as compared to HF0 indicating decrease in its crosslinking density.

Thus, the results of carboxyl content and instrumental characterization were in complete agreement with each other.

3.6. Tensile strength of hydrogel films

The tensile strength of the hydrogel films was found to be increased with increase in the concentration of PVA up to 0.2% followed by decrease (see Table 2). The reason behind low value of tensile strength of CMC hydrogel films may be the lesser extent of crosslinking. The addition of PVA not only increases the extent of crosslinking but also enhances the hydrogen bonding interaction in between unreacted OH groups of PVA and, OH and carbonyl groups of other components (i.e.

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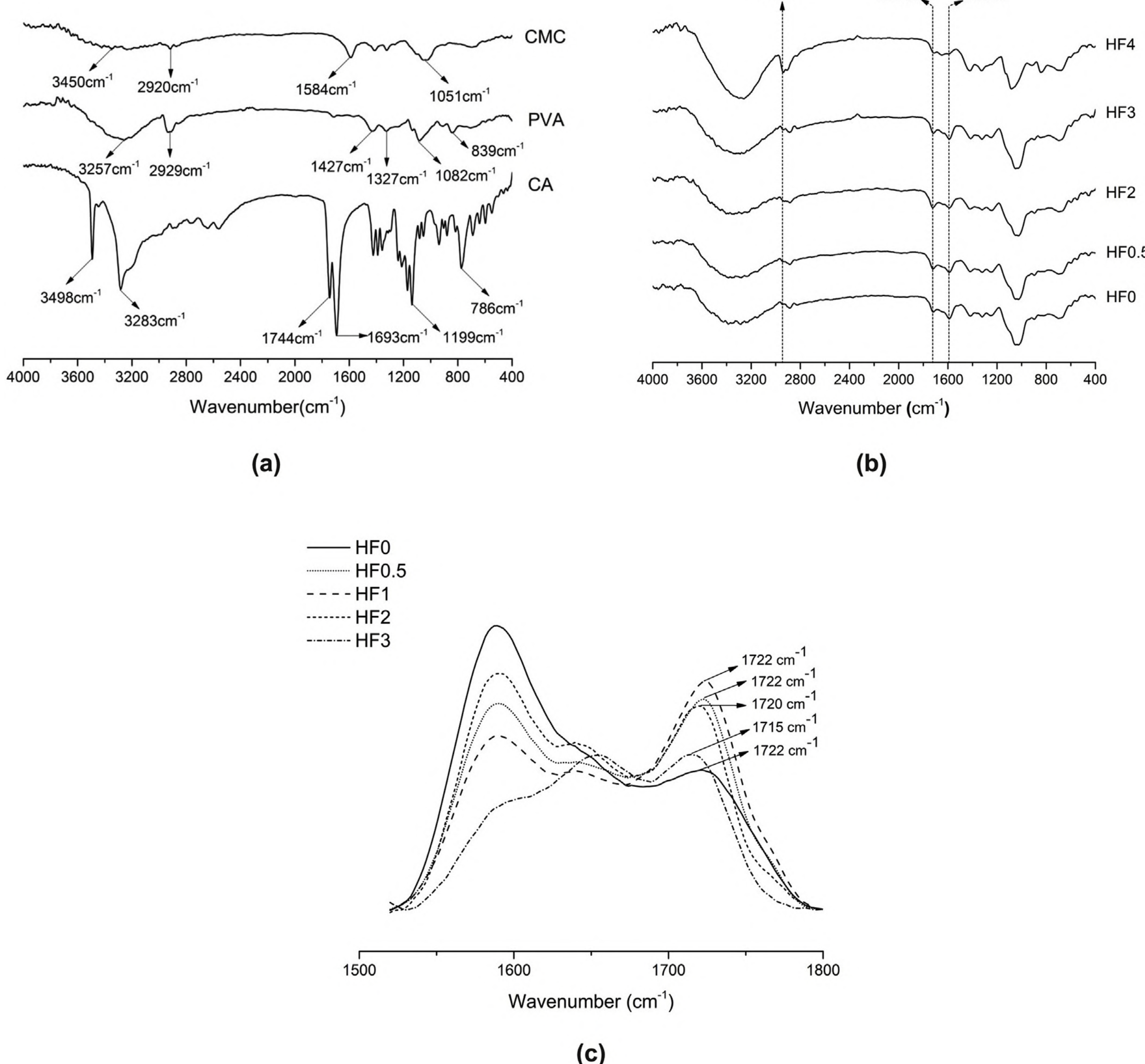

Fig. 3. ATR-FTIR spectra of CMC, PVA and CA (a), hydrogel films (b) and overlain spectra (baseline corrected) of hydrogel films in the region $1500\,cm^{-1}$ to $1800\,cm^{-1}$ (c).

CMC and CA crosslinks) within the CMC-PVA hydrogel films [46]. This can improve the mechanical property of the hydrogel films. The batch HF1 exhibited maximum tensile strength ($3.54 \pm 0.12\,kg/mm^2$) than the other hydrogel films. As the concentration of PVA was increased above 0.2%, the tensile strength of the CMC-PVA hydrogel films was found to be reduced slightly which can be attributed to the decrease in the crosslinking density of the hydrogel films. As compared to the pure CMC hydrogel film, CMC-PVA hydrogel films exhibited better mechanical strength.

3.7. Swelling study

Fig. 5 illustrates the swelling behavior of the hydrogel films. All the films showed initial increase in the swelling ratio till equilibrium followed by erosion. The pure CMC hydrogel films (HF0) showed minimum equilibrium swelling ratio (ESR) as compared to the CMC-PVA hydrogel films (HF0.5-HF3) (see Table 2). It was noted that despite of greater extent of crosslinking than HF0, HF0.5 and HF1 showed good swelling. This can be related to the increase in the total polymer concentration where PVA chains will be arranged in between the CMC chains. Secondly, on addition of PVA, the number of free OH groups in the hydrogel film will increase which can enhance the hydrophilicity of the hydrogel films [47]. An increase in the number of unreacted hydroxyl groups can be confirmed from an increase in the intensity of O-H stretching vibrations from HF0.5 to HF3 (see Fig. 3b). The other reason behind increase in the swellability of HF0.5 and HF1 than HF0 may be introduction of highly hydrophilic carboxylic groups due to increase in the number of crosslinks. These free carboxylic groups play an important role in the formation of a polyelectrolyte network within the hydrogel films and improve the water sorption [19]. The time taken by HF1 to reach the equilibrium (5 h) was found to be more than HF0 (4 h) which can be due to greater crosslinking density in HF1. A further increase in the concentration of PVA increased the swellability of the hydrogel films (HF2 and HF3) and also reduced the time required to achieve the equilibrium. This may be due to increase in the hydrophilicity and decrease in the crosslinking density of HF2 and HF3. HF3 showed maximum ESR than the other films (see Table 2).

3.8. GTM loading and release

The GTM loading increased with increase in the swellability of the hydrogel films and hence, the amount of PVA (see Table 2). The greater

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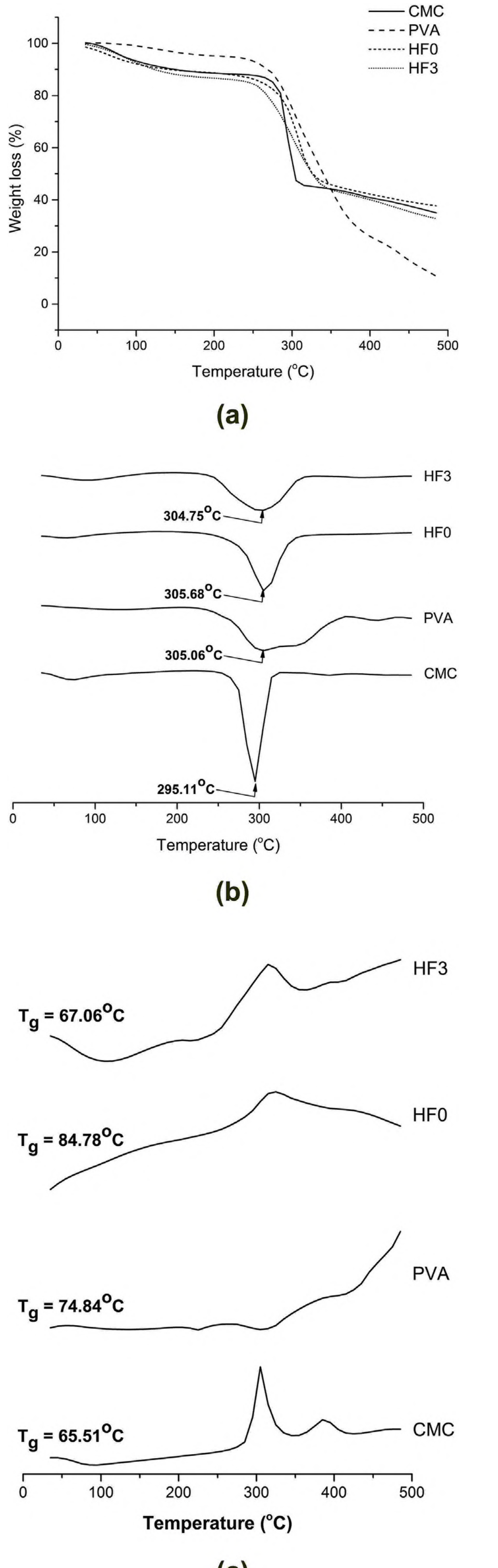

Fig. 4. TGA (a), DTG (b) and DSC (c) thermograms of CMC, PVA and hydrogel films (HF0 and HF3).

the swellability of the hydrogel films more will be their tendency to absorb the drug solution. As a result, large number of drug molecules will get entrapped in the polymeric network of hydrogels. The CMC-PVA hydrogel films showed significant improvement ($p < 0.05$) in the GTM loading than the CMC hydrogel film, with HF3 exhibiting maximum drug loading.

Besides swellability, hydrogen bonding interaction in between amine groups of GTM and hydrogel components (hydroxyl and carbonyl groups), and electrostatic interaction in between protonated amine groups of GTM and deprotonated carboxyl groups of hydrogel films may also contribute in enhancing the loading of GTM. In order to confirm the hydrogen bonding interactions, ATR-FTIR analysis of pure GTM and GTM loaded HF3 was performed (see Fig. 6). The spectrum of GTM showed absorption bands in the range of 2900–3500 cm^{-1} corresponding to N-H stretching vibrations of amino groups. Multiple peaks were found to be fused in the range of 2300–2900 cm^{-1} which represented C-H stretching and absorption due to the other components of GTM [48]. The peaks in the range of 1400–1700 indicated N-H bending, C-H stretching and C-N stretching vibrations. The strong band in the range of 900–1250 was related to C-N and C-O stretching vibrations. In spectrum of GTM loaded HF3, the N-H stretching bands of GTM nearly disappeared. Also, the broad peak of blank HF3 representing O-H stretching shifted from 3284 cm^{-1} to 3217 cm^{-1}, with decrease in its intensity. Such changes suggest the existence of hydrogen bonding interactions in between NH_2 groups of GTM and OH groups of HF3. As the number of OH groups increase from HF0 to HF3, the hydrogen bonding interactions in between GTM and hydrogel components will increase, thus enhancing the loading of GTM in the hydrogel films. The hydrogen bond formation is also possible in between amine groups of GTM and carbonyl groups of hydrogel films. The C-O stretching peak of HF3 at 1082 cm^{-1} shifted to lower wavenumber due to peak of GTM at 1037 cm^{-1}. The other peaks of GTM overlapped with the peaks of HF3 and therefore could not be distinguished.

Furthermore, electrostatic interaction in between GTM and free COOH groups of hydrogel films was possible because the drug loading was carried out in the aqueous drug solution of pH 5.8. At this pH, the amine groups of GTM undergo protonation (pKa = 8.6) whereas free carboxylic acid groups of hydrogel films are deprotonized. Consequently, electrostatic attraction will occur in between NH_3^+ groups of GTM and COO^- groups of hydrogel films, thus assisting in the drug loading improvement. As CMC-PVA hydrogel films exhibited greater carboxyl content than the pure CMC hydrogels, such electrostatic interaction will be more favored in their case.

An increase in the GTM loading in the CMC-PVA hydrogel films inspite of decrease in their carboxyl content indicates that the drug loading was mainly dependent upon swellability and hydrogen bonding.

Fig. 7 demonstrates the drug release profile of the GTM loaded hydrogel films. HF0 showed a noticeable burst release of GTM (26.96 ± 1.48%) within first hour, followed by rapid release. During drying of the drug loaded hydrogel films, most of the drug molecules diffuse along with the solvent towards the surface of the hydrogel films. As a result, a large number of drug molecules concentrate near the surface of the hydrogel films resulting into burst release. An increase in the concentration of PVA reduced the burst release due to an increase in the interaction between GTM and PVA (hydrogen and electrostatic bonding) within the hydrogel films. This will lead to maximum entrapment of GTM within the core of hydrogel matrix and concentration of the GTM molecules near the hydrogel surface will be reduced, thus reducing the burst release in case of CMC-PVA hydrogel films.

In case of HF0, nearly complete GTM release (98.90 ± 1.74%) occurred in 12 h. The CMC-PVA hydrogel films were capable of controlling the release of GTM above 24 h. The retardation of drug release was observed as we move from HF1 to HF3 which can again be attributed to the intermolecular interactions which can restrict the rapid transit of the GTM molecules from the hydrogel matrix into the release

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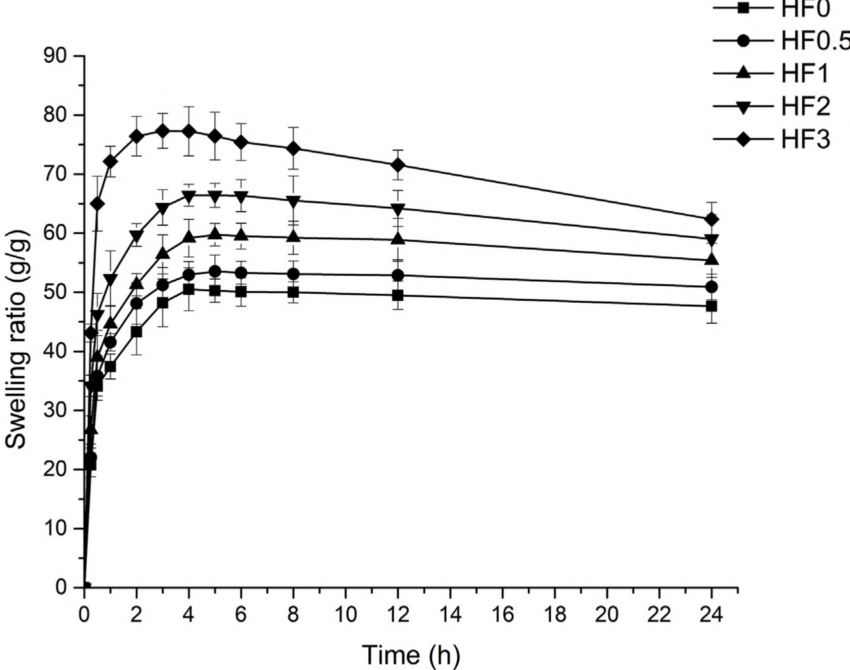

Fig. 5. Swelling profile of the hydrogel films (HF0-HF3).

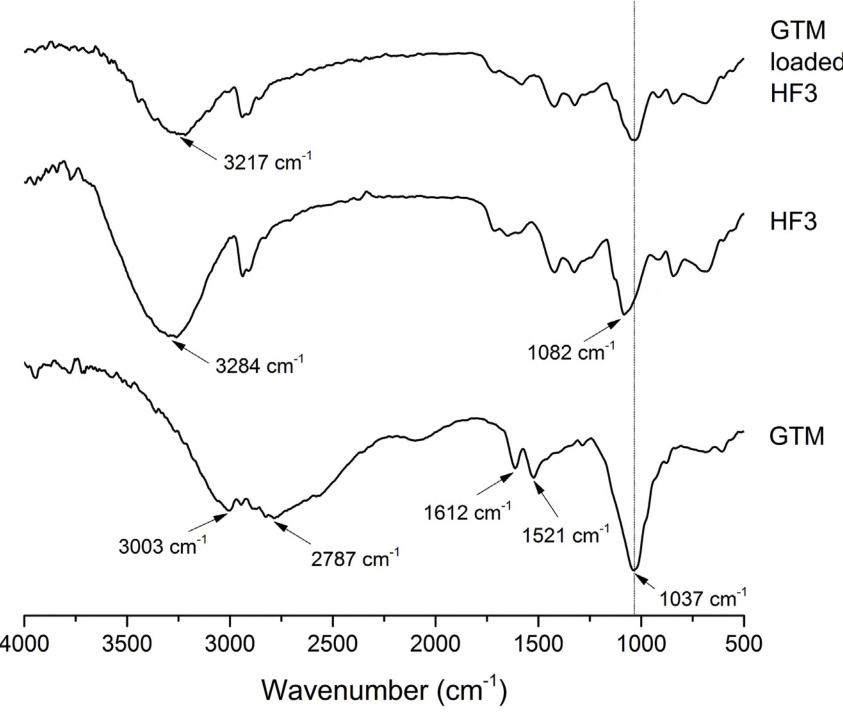

Fig. 6. ATR-FTIR spectra of GTM, HF3 and GTM loaded HF3.

medium. HF3 showed maximum drug release retardation (80.29 ± 2.22% at the end of 24 h) as compared to HF0.5 (98.41 ± 2.14% at the end of 24 h), HF1 (94.03 ± 2.86% at the end of 24 h) and HF2 (86.54 ± 1.94% at the end of 24 h). As HF3, showed maximum drug loading and had greater tendency to control the release of GTM than the other batches, it can be considered as optimized hydrogel film.

3.9. Hemocompatibility

The results of hemolysis assay are shown in Table 2. It was observed that the hemolysis due to pure CMC and CMC-PVA hydrogel films was less than 5% indicating hemocompatible nature of the hydrogel films [7]. From the values of percent hemolysis, it was difficult to determine the effect of PVA on the extent of hemolysis.

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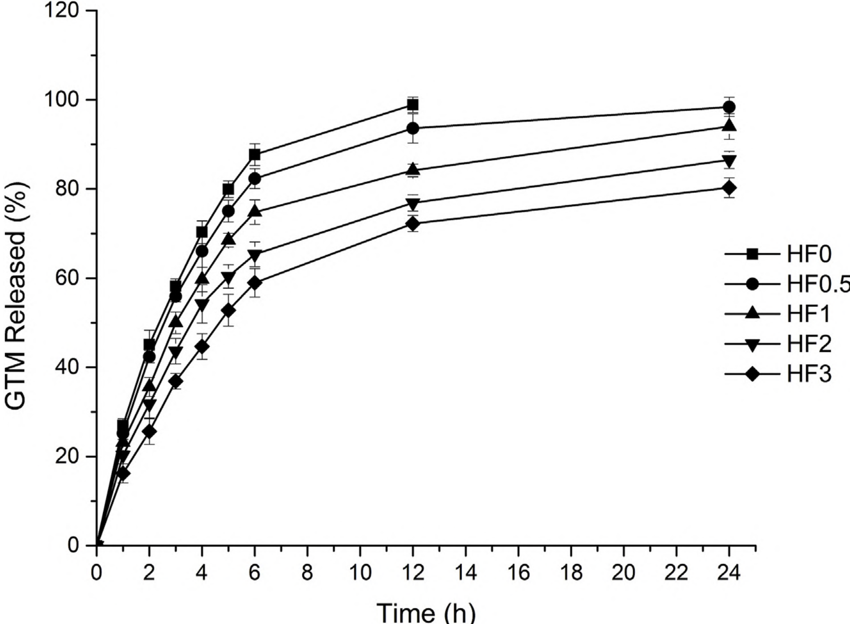

Fig. 7. GTM release (%) from the pure CMC and CMC-PVA hydrogel films.

4. Conclusion

The CA crosslinked CMC-PVA hydrogel films were formed by esterification-crosslinking mechanism. As the concentration of PVA in the feed was increased, the carboxyl content increased initially indicating increase in the crosslinking density, followed by decrease due to dilution effect. The presence of ester crosslinks and extent of crosslinking was confirmed from ATR-FTIR, solid state ^{13}C NMR and thermal analysis. The CMC-PVA hydrogel films showed good mechanical strength than the pure CMC hydrogel films. An increase in the amount of PVA in the hydrogel films improved the swellability of the hydrogel films and also enhanced the loading of GTM. CMC-PVA hydrogel films were capable of extending the release of GTM above 24 h. The prepared films were hemocompatible. Thus, CA crosslinked CMC-PVA hydrogel films exhibited potential to be used as an efficient and cheap biomaterial for delivery of water soluble basic drugs; however this can be confirmed only after conduction of few more tests such as MTT assay and in *vivo* studies.

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Conflicts of interest

Authors declare no conflict of interest.

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Original Article

Design, Development and Evaluation of Self Nanoemulsifying Drug Delivery System of Garlic Oil using Capryol PGMC

Priyanka Sangar[1], Bandgar Sandip[1,*], Shelake Sardar[2], Patil Pravin[1], Bhagwat Durgacharan[3], Patil Shitalkumar[1]

[1]Department of Pharmaceutics, Ashokrao Mane College of Pharmacy, Peth-Vadgaon, Kolhapur, Maharashtra, INDIA.
[2]Department of Pharmaceutics, Ashokrao Mane Institute of Pharmacy, Ambap, Maharashtra, INDIA.
[3]Department of Pharmaceutics, Bharati Vidyapeeth College of Pharmacy, Kolhapur, Maharashtra, INDIA.

ABSTRACT

Introduction: At present days there was considerable attention has been taken to develop lipid based pharmaceutical preparation which improves solubility as well as permeability leads to improve oral bioavailability of poorly water soluble drug with a system known as self nano-emulsifying drug delivery system. **Materials and Methods:** The SNEDDS of garlic oil was prepared by using oleic acid as oil, capryol PGMC as a surfactant and ethanol as a co-surfactant, as the garlic oil shows better solubility in these excipients which is find out by constructing pseudo-ternary phase diagram. The Km = 3 was selected for the preparation of SNEDDS of garlic oil because it shows better nanoemulsion region as compared to Km = 1 and 2. **Discussion:** The formulated SNEDDS of garlic oil was evaluated for physical characterization, thermodynamic stability, rheology study, globule size and zeta potential, dispersibility study, cloud point determination, % transmittance, drug content, FTIR study and *in vitro* drug release study. Three batches of SNEDDS of garlic oil was formulated using Km value 3 which cover maximum nanoemulsion region, containing oleic acid (solubility 57.53 ± 0.45), Capryol PGMC (solubility 59.80 ± 0.82) and ethanol (solubility 49.83 ± 0.30). Based on the compatibility study, optimum globule size (177.2 nm), minimum polydispersity (0.386), higher drug content (90.89 ± 0.68) and higher drug release (98.85%), batch F2 was optimized. **Conclusion:** The bioavailability problem can be overcome by the Self nano- emulsifying drug delivery system, which presents the more drug in solubilized form in the body as compared with other conventional drug delivery systems.

Key words: Self Nanoemulsifying Drug Delivery System, Garlic oil, Pseudo ternary phase diagram, Capryol PGMC, poorly water soluble drug.

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Correspondence:
Mr. Sandip Akaram Bandgar,
Department of Pharmaceutics, Ashokrao Mane College of Pharmacy, Peth-Vadgaon, Kolhapur, Maharashtra-416112, INDIA.
Phone: +91 8600009719
E-mail: bandgarsandip21@gmail.com

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INTRODUCTION

Garlic, botanically known as *Allium sativum* Linn. a member of Liliaceae family is one of the earliest documented example of plants employed for the treatment of diseases and maintenance of health.[1] Garlic oil is best known for its number of medicinal values such as anti-atherosclerosis, blood lipid and sugar modulation, antifungal, antimicrobial, anti-thrombolic, cardiovascular disease treatment and stimulation of immune system.[2] However, the application of garlic oil in the food industry is limited due to its volatility, strong odour, insolubility in water and low physicochemical stability.[3] To overcome these problems various methods are listed in the literature which include incorporation of hydrophilic excipients, solid dispersion, micellar solubilization, microemulsion etc. But in recent years considerable attention has been made to develop lipid based pharmaceutical preparation as it improves not only solubility but also permeability which leads to improve oral bioavailability of poorly water soluble

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drugs, such a system is known as Self Nanoemulsifying Drug Delivery System (SNEDDS).[4]

Self Nanoemulsifying Drug Delivery Systems (SNEDDS) are regarded as anhydrous forms of the nanoemulsion. SNEDDS are homogenous liquid mixtures consisting of drug, natural or synthetic oil, surfactant and co-surfactant that have a rival ability of spontaneously forming fine oil-in-water (O/W) nano-emulsions of size about 200 nm or less, upon dilution with water. These preparations are thermodynamically stable and transparent or translucent system. Nano-sized dispersion of nanoemulsion was stabilized by the addition of surfactants and co-surfactants. SNEDDS are also known as nanoemulsion, miniemulsion, ultrafine emulsion or submicron emulsion. These systems were formulated mainly by using medium chain triglycerides, oils and non-ionic surfactant, which is important in oral ingestion. SNEDDS are one of the stable nanoemulsion and it provides a large interfacial area for partitioning of drug between oil and aqueous phase, thereby improves the rate of drug dissolution and increases bioavailability of the drug formulation. SNEDDS are the most preferred drug delivery system due to their stability, practicability of easy oral administration and ability to enhance drug self emulsification inside the gut.[5,6]

Thus, utilizing SNEDDS as a promising technology to overcome the problems of low bioavailability leads to develop a drug with improved solubility as well as improved physiochemical stability.[7] Hence, SNEDDS of garlic oil will control different aspects of drug efficacy such as pharmacokinetics, bioavailability, targeted delivery, non-specific toxicity and immunogenicity and will be beneficial as suitable dosage form which results in better patience compliance and improved therapeutics.[8,9]

MATERIALS AND METHODS

Garlic oil (Sanket Enterprises, Mumbai), Oleic acid (Molychem, Mumbai), Capryol PGMC (Gattefosse, France), Ethanol and Methanol (S. D. fine Chemicals, Mumbai). All other materials or chemicals used were of analytical grade.

Selection and screening of drug components

For the selection of suitable components with good solubilizing capacity for garlic oil, saturation solubility of garlic oil was examined in various oils (oleic acid, cotton seed oil, almond oil, castor oil), surfactant (Capryol PGMC, Labrafac PG, tween 20, span 80, cremophore EL) and co-surfactants (Ethanol, propylene glycol, PEG 200, glycerol). In this solubility study the excess amount of drug i.e. garlic oil was added into screw caped glass vials containing two ml of each excipients followed by sealed vials. The sealed vials were kept in sonicator for 2 h. after that the mixture was kept in water bath at 40°C for 24 h and then these vials were centrifuged at 15000 rpm for 30 min. The samples were collected and filtered using a membrane filter (0.45 micro meter). The filtrate was suitably diluted with methanol and drug concentration was obtained by using UV Visible spectrophotometer.[10]

Construction of pseudo ternary phase diagram

The pseudo ternary phase diagram was constructed without garlic oil to recognize the maximum self-emulsifying domain existence and to specify the optimal ratio of oil, surfactant and co-surfactant for the SNEDDS formulations. The pseudo ternary phase diagrams were constructed by drop wise addition of distilled water to homogeneous liquid mixture of oil, surfactant and co-surfactant, at ambient temperature by water titration method.

From result of solubility studies and screening of solubility of excipient: Oleic acid, Capryol PGMC and ethanol were selected as oil, surfactant and co-surfactant. The mixture of oil and surfactant / co-surfactant (S/CoS) i.e S_{mix} at certain weight ratio were diluted with water in drop wise addition. Surfactant and co-surfactant mixture were mixed in different weight ratio at different Km value 1, 2, 3 ratio i.e. 1:1, 2:1, 3:1 (w/w). The oil and S_{mix} were mixed at ratio of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 Figure 2, 3 and 4. Slow titration with aqueous phase was done to each ratio of oil and S_{mix} and visual observation was carried out for transparency and flowability of nanoemulsion. The mixtures were examined for turbidity to transparency. Clear and isotropic mixtures were deemed to be within Nano emulsion region. On the other hand, the emulsion with coarse droplets or temporary emulsion exhibiting coalescence or creaming on terminating stirring was considered "bad". All the tests were performed in triplicate.[11,12]

Preparation of liquid SNEDDS

The phase diagram was constructed at different Km values. The Km value at which nano-emulsion region obtained was selected for further studies. Three formulations were selected from this nano- emulsion region.

Oil, surfactant and co-surfactant were accurately weighed and mixed by gentle stirring. Based on solubility, formulation amount of garlic oil (100mg) was dispersed into mixture of oil and surfactant and co-surfactant. All the components were mixed by gentle stirring on

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Table 1: Composition of selected formulation.				
Batch code	Drug (mg)	Smix (ml)	Oil (ml)	Water (ml)
Garlic F1	100	30	10	60
Garlic F2	100	40	10	50
Garlic F3	100	50	10	40

magnetic stirrer until garlic oil was completely dissolved. Mixture was sealed in glass vial and stored at room temperature for further study.[13] The composition of selected formulations showed in Table 1 and Figure 6.

Evaluation of SNEDDS[14-16]

Physical characterization

The organoleptic properties of the SNEDDS such as, color, odor and physical state were checked by visual observation.

Thermodynamic stability study

The thermodynamic stability of lipid based formulation can be adversely affected by precipitation of the drug in the excipients matrix. This can be also lead to phase separation of the excipients affecting not only formulation performance as well as visual functioning. The thermodynamic stability study was based on following three tests:

Heating and cooling cycle

Three heating/cooling cycles between 4°C and 40°C with storage at each temperature for not less than 24 h. The resultant formulations were evaluated for their thermodynamic instability like precipitation and phase separation. The formulation which qualifies this test was subjected to further study.

Centrifugation study

The prepared formulations were centrifuged using laboratory centrifuge at 5000 rpm for 30 min. The resultant formulations were then determined for any instability problem, such as phase separation, cracking or creaming. A formulation which qualifies this test subjected for further study.

Freeze thaw cycle

To determine the stability of SNEDDS freeze thawing was employed. The prepared formulations were subjected to three freeze thaw cycles, which included freezing at −4°C for 24 h followed by thawing at 40°C for 24 h. Then centrifugation was performed at 3000 rpm for 10 min. Then the tested formulations were observed for phase separation.

Rheological study

The viscosity of the prepared formulations was determined by using Brookfield viscometer which determines the consistency of nano-emulsion formulation. 1ml of each prepared formulations were diluted 10 times with distilled water and then viscosity was measured using Brookfield viscometer and assessed visually for any phase separation.

Globule size and zeta potential determination

Droplet size of SNEDDS was determined by photon correlation spectroscopy that analyses the fluctuations in light scattering due to Brownian motion of the particle, using a Zetasizer. The zeta potential of the SNEDDS should be evaluated as it may further give an idea of the colloidal stability. Both these tests were carried out by using Nanoparticle analyzer sz-100 (Horiba Scientific, Japan).

Dispersibility test (Assessment of self emulsification)

The efficiency of self- emulsification of oral nanoemulsion is determined by using a standard USP XXII dissolution apparatus II. 1ml of each formulation is added to 500 ml of water at 37±0.5°C. The stainless steel dissolution paddle rotating at 50 RPM provided gentle agitation. The emulsification time assessed visually.

Percent transmittance

The percent transmittance of the prepared formulations were measured using UV Visible double beam spectrophotometer or Single Beam Spectrophotometer using distilled water as blank at suitable wavelength. For this study 1ml of each prepared formulations were diluted to 100 ml of distilled water and observed for any turbidity and % transmittance was observed by using UV-visible spectrophotometer (Shimadzu UV 1800) against distilled water at suitable wavelength.

Cloud point determination

The prepared formulations were diluted with distilled water in the ratio 1:250, placed in water bath and its temperature was increased gradually. Cloud point was measured at the temperature at which there was a sudden appearance of cloudiness occurred.

Drug content

The total amount of drug in the formulation was analyzed by dissolving the formulation in 10 ml of

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methanol. This solution was vortexed for 10 min in vortex mixture. The mixture was centrifuged at 15,000 rpm for 10 min. Then the supernatant was filtered through Whatman filter paper. The concentration of garlic oil was analyzed spectrophotometrically at 306 nm.

FTIR Study

The prepared formulations were analyzed by Fourier Transform infrared spectroscopy (UV Agilent Technology) to characterize the probable structural modification produced. The sample was analyzed in the region of 4000 and 400 cm^{-1} and then sample or mixture kept into sample holder for analysis.

In vitro drug release study

In vitro dissolution studies of prepared formulations were carried out. The prepared formulations were filled in hard gelatin capsule. *In vitro* drug release profile of garlic oil from SNEDDS was assessed using USP dissolution testing apparatus I (basket type) at 50 rpm with 900 ml 0.05 M NaCl of pH 1.5 as dissolution medium. Temperature was set at 37. 0± 0.5°C and sampling interval were fixed at 5, 10, 15, 20, 25, 30 min.1ml of sample withdraw at each time interval and replaced with 1ml fresh 0.05M NaCl of pH 1.5 solution. The solution was immediately filtered through whatman filter paper and the filtrate was diluted with dissolution medium up to 10ml and evaluated for the drug content using UV-Visible spectrophotometric method at 306 nm.[17]

RESULTS AND DISCUSSION

From the solubility study oleic acid was selected as oil, capryol PGMC as surfactant and ethanol as co-surfactant, as the garlic oil shows more solubility than the other components which were shown in Figure 1.

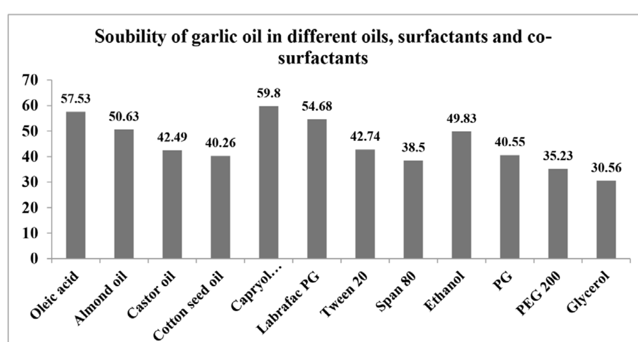

Figure 1: Solubility of garlic oil in different oils, surfactants and co- surfactants.

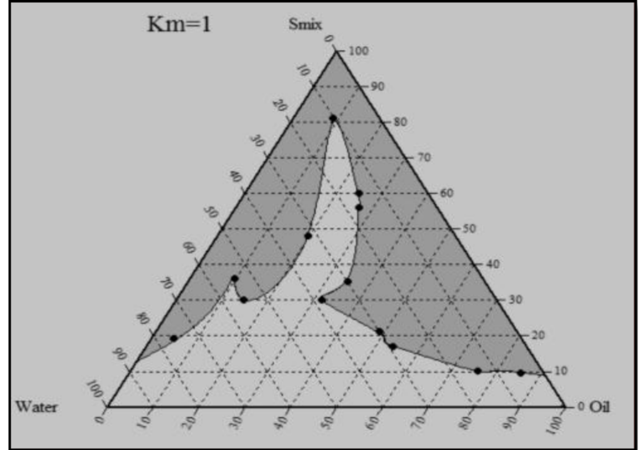

Figure 2: Ternary phase diagram of Oleic acid, Capryol PGMC, Ethanol at Km=1.

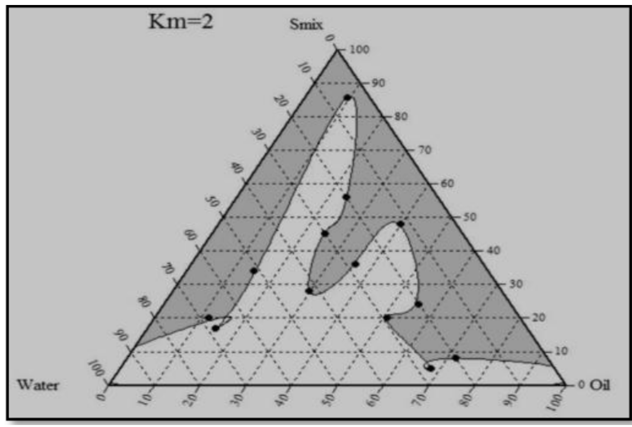

Figure 3: Ternary phase diagram of Oleic acid, Capryol PGMC, Ethanol at Km=2.

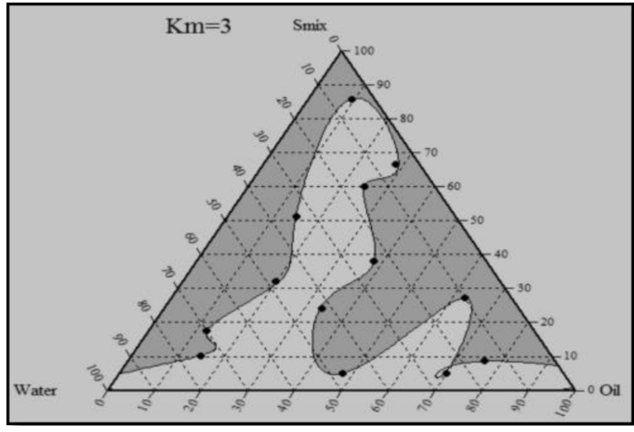

Figure 4: Ternary phase diagram of Oleic acid, Capryol PGMC, Ethanol at Km=3.

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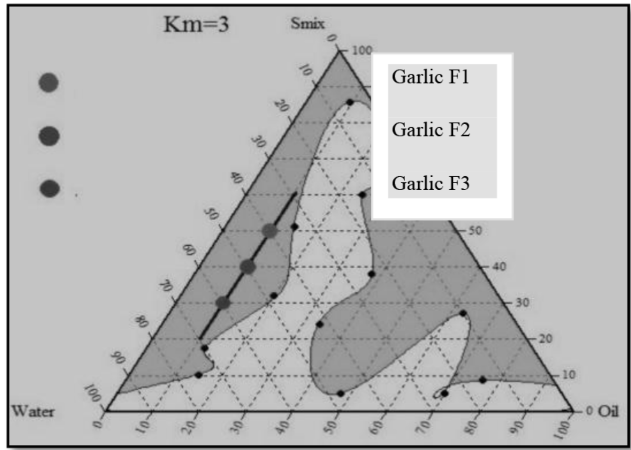

Figure 5: Selected composition of formulations Garlic F1 to Garlic F3.

Figure 6: Formulated batches of SNEDDS of Garlic oil.

Construction of pseudo-ternary phase diagram

Preparation of liquid Self Nano-emulsifying Drug Delivery System

Oleic acid-Capryol PGMC-Ethanol-Water based system selected at final Pseudoternary phase diagram of various surfactants and co-surfactant weight ratio was constructed and system of highest water absorption (highest nano emulsion region) selected for formulation. The phase diagram at Km value 3 showed better nano-emulsion existence region than 1 and 2.

Three formulations were selected from phase diagram at Km value 3, named as Garlic F1, Garlic F2, Garlic F3, as shown in Figure 5. Quantitative unit compositions of selected formulation of SNEDDS were presented in Table 2.

Evaluation of prepared SNEDDS

Physical characterization

The physical characterization of formulated batches was shown in Table 3.

Table 2: Composition of selected formulations.

Batch	Drug (mg)	Smix (ml)	Oil (ml)	Water (ml)
F1	100	30	10	60
F2	100	40	10	50
F3	100	50	10	40

Table 3: Physical characterization of formulated batches.

Sr. No.	Parameters	Result
F1	Physical state	Liquid
F2	Color	Light yellow
F3	Taste	Characteristic

Table 4: Thermodynamic stability study of formulated batches.

Batch	Heating cooling cycles	Centrifugation test	Freeze thaw cycles
Garlic F1	+	+	+
Garlic F2	+	+	+
Garlic F3	+	+	+

Thermodynamic stability study

Thermodynamic stability of SNEDDS was essential to its performance, which can be affected by precipitation of the drug. In addition the formulation having poor physical stability can affects the formulation performance and it also leads to phase separation. Hence thermodynamic stability studies were performed by performing heating cooling cycle, centrifugation test and freeze thaw cycle, it was observed that formulation passed the heating cooling cycle test, hence further exposed to centrifugation test then it was taken for freeze thaw stress test. After freeze thaw stress test it was found that all three formulations showed good stability with no phase separation, creaming or cracking were showed in Table 4.

Rheological study

The rheological properties of the prepared formulations were evaluated by Brookfield viscometer. This viscosities determination confirm the system is o/w or w/o. If system has low viscosity then it is o/w and high viscosity then w/o. Viscosity of prepared batches was determined by diluting 1 ml sample of each batch with 10 ml and 100 ml of distilled water by using Brookfield viscometer. The obtained results were showed in Table 5.

Globule size and zeta potential determination

The globule size of the emulsion is a crucial factor of self nano-emulsification performance because it deter-

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mines the rate and extends of drug release as well as drug absorption. Also, smaller particle size of the emulsion droplets may lead to more rapid absorption and improve the bioavailability.

The globule size and zeta potential determined using Nanoparticle analyzer sz-100. The average globule size was taken into consideration. Table 6 shows the particle size, zeta potential and PDI of formulated batches of garlic oil SNEDDS diluted with water. The average particle size obtained from optimized batch Garlic F2 of SNEDDS formulation of garlic oil was found to be 177.2 nm, zeta potential -25 mv and polydispersity index was found to be 0.386 Figure 7 and 8. Zeta potential is the another property that was assessed for increased absorption of SNEDDS is the charge of oil droplets which is usually found to be negative due to the presence of free fatty acid. These results indicate that the optimal garlic oil SNEDDS formulation produced clear nano emulsion with nanometric size.

Dispersibility test (Assessment of Self Emulsification)

Emulsification time is a major parameter that helps in the determination of emulsification rate of SNEDDS. Oil is a major factor that affect relatively because when it present in high concentration, it prevent penetration of water. While hydrophilic compound such as surfactant and co-surfactant helps in dispersion and so enhance the emulsification rate. The efficiency of self- emulsification could be estimated primarily by determining the rate of oil droplets of SNEDDS formulation dispersed quickly and completely when subjected to aqueous dilution under agitation. The self- emulsification time of prepared formulation of SNEDDS were show in Table 7.

Percent transmittance

The results of % transmittance were shown in Table 8. The clarity of prepared nano emulsion was checked by transparency, measured in terms of transmittance. SNEDDS forms o/w nano emulsion since water is external phase. Formulation Garlic F2 has 97.50 % transmittance. The result indicates good clarity of emulsion Table 8.

Cloud point determination Cloud point of prepared nanoemulsion was found to be higher than 80°C, which indicate that nanoemulsion will be stable at physiological temperature without risk of phase separation. The obtained results were showed in Table 9.

Drug content The drug content of the prepared formulations was shown in Table 10.

Table 5: Viscosity determination of formulated batches.

Sr. No.	Batch	Viscosity Cp	
		10 ml dilution	100 ml dilution
1	Garlic F1	0.5467	0.3589
2	Garlic F2	0.4043	0.3467
3	Garlic F3	0.5689	0.3654

Table 6: Globule size, Zeta potential and PDI of prepared formulations.

Sr. No.	Batch	Average Particle size (Droplet size /Globule size	Zeta potential	Poly-dispersity index (PDI)
1	Garlic F1	185.00 nm	-20 mv	0.567
2	Garlic F2	177.2 nm	-25 mv	0. 386
3	Garlic F3	193.90 nm	-18 mv	0.690

Table 7: Self-emulsification time of prepared formulation.

Sr. No.	Batch	Emulsification time (sec)
1	Garlic F1	59.83 ± 0.76
2	Garlic F2	70.30 ± 0.49
3	Garlic F3	60.36 ± 0.28

Table 8: % Transmittance of prepared formulations.

Sr. No.	Batch	% Transmittance
1	Garlic F1	92.71 ± 0.25
2	Garlic F2	97.50 ± 0.40
3	Garlic F3	95.33 ± 0.41

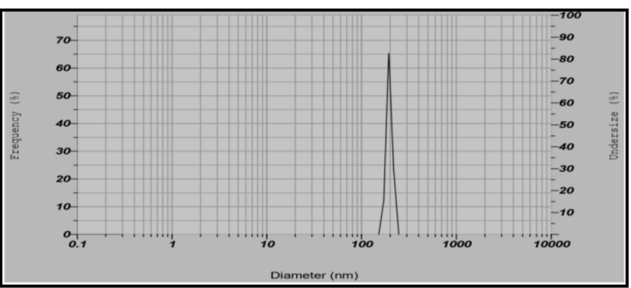

Figure 7: Globule size analysis of optimized batch Garlic F2.

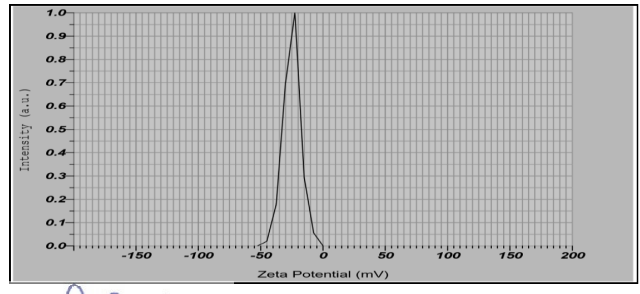

Figure 8: Zeta potential of optimized batch Garlic F2.

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Table 9: Cloud point determination of prepared formulation

Sr. No.	Batch	Cloud point
1	Garlic F1	More than 80°C
2	Garlic F2	More than 95°C
3	Garlic F3	More than 90°C

Table 10: Drug content of prepared formulations.

Sr. No.	Batch	% Drug content
1	Garlic F1	75.05 ± 0.55
2	Garlic F2	90.89 ± 0.68
3	Garlic F3	67.98 ± 0.75

Table 11: % Drug release of prepared formulations.

Sr. No.	Batch	% Drug release
1	Garlic F1	85.78
2	Garlic F2	98.85
3	Garlic F3	90.78

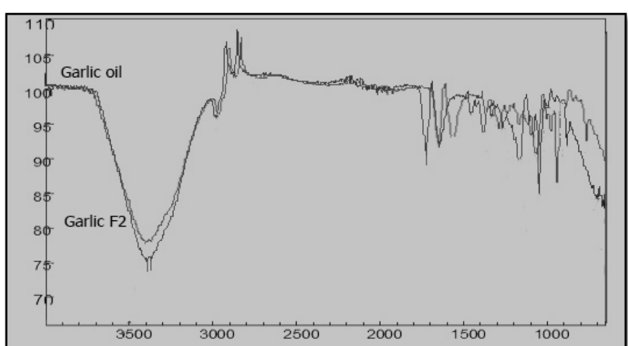

Figure 9: FTIR Spectra of garlic oil and optimized batch F2.

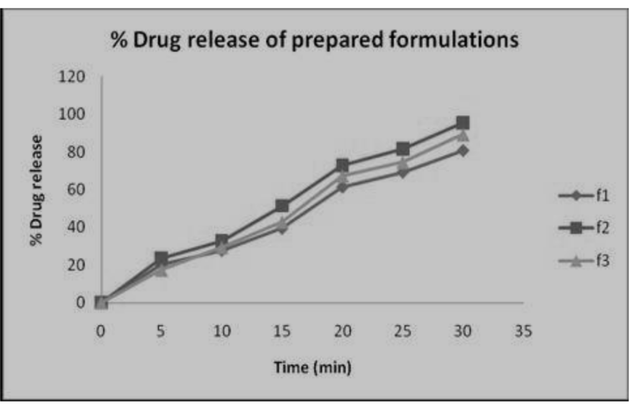

Figure 10: % Drug release of prepared formulations.

FTIR study

Drug and formulation has shown no any difference in spectra indicate drug is intact in the formulation which was shown in Figure 9.

In vitro drug release

In vitro drug release study of prepared formulations of garlic oil SNEDDS was performed in 0.05 M NaCl of pH 1.5. The % drug release was shown in Table 11 and Figure 10.

CONCLUSION

In this study, liquid SNEDDS was formulated by using capryol PGMC as surfactant. From this study, it was concluded that the prepared liquid SNEDDS was thermo dynamically stable with good self-emulsification efficiency, improved dissolution rate and having globule size in the nanometric range which may be physiologically stable. The SNEDDS with relatively high drug content was prepared which self-emulsified easily with mean emulsion droplet size of 177.2 nm. Thermodynamic stability study and cloud point study confirmed that the SNEDDS had no dilution effect and was stable without any precipitation of drug and without any change in emulsion droplet size.

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The authors are thankful to Ashokrao Mane College of Pharmacy management for providing facility to carry out research work, also thankful to Sanket Enterprises, Mumbai for providing Garlic Oil and Gatteffosse Mumbai for providing Capryol PGMC as gift sample.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

FTIR: Fourier Transform Infrared Spectrometer; **SNEDDS:** Self Nanoemulsifying Drug Delivery System; **RPM:** Revolutions per Minute; **PGMC:** Propylene Glycol Monocaprylate.

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PICTORIAL ABSTRACT

Problems associate with Garlic oil:
Volatility, Strong odour, Insolubility in water, Low physicochemical stability

SUMMARY

In this present work, liquid SNEDDS of garlic oil was formulated by using capryol PGMC as surfactant. From this study, it was observed that the prepared liquid SNEDDS of garlic oil was thermodynamically stable with good self-emulsification efficiency, improved dissolution rate and having globule size in the nanometric range which may be physiologically stable. The garlic oil shows better solubility in oleic acid (oil), capryol PGMC (surfactant) and ethanol (co-surfactant) which was find out by constructing pseudo-ternary phase diagram. The Km=3 was selected for the preparation of SNEDDS of garlic oil because it shows better nanoemulsion region as compared to Km=1 and 2. The SNEDDS with relatively high drug content was prepared with mean emulsion droplet size of 177.2 nm. Thermodynamic stability study and cloud point study confirmed that the SNEDDS had no dilution effect and was stable without any precipitation of drug and without any change in emulsion droplet size.

About Authors

Ms. Priyanka C. Sangar, has completed B Pharm and M Pharm in Pharmaceutics from Ashokrao Mane College of Pharmacy, Peth Vadgaon in 2018. Currently, he is working as Assistant Professor at Yashoda Technical Campus, Satara.

Mr. Sandip A. Bandgar, has completed B Pharm in 2004 and M. Pharm (Biopharmaceutics) in 2008 from Government College of Pharmacy, Karad and pursuing his Ph.D from Shivaji University, Kolhapur. Presently, he is working as Assistant Professor and PG Teacher, Department of Pharmaceutics, Ashokrao Mane College of Pharmacy, Peth Vadgaon. He has 2 years of diploma and 11 years of degree teaching experience. He has published 10 research articles in reputed national and international journals and 4 books on his credit. He has presented various research papers in national and international level conferences and workshops. He is a life member of various professional associations like APTI, MSPC and KPSF.

Mr. Sardar S. Shelake, has completed M.Pharm from Ashokrao Mane College of Pharmacy, Peth Vadgaon in Pharmaceutics. He has qualified GPAT in 2013. Presently he is perusing Ph.D. in Shivaji University, Kolhapur. Currently, he is working as Assistant Professor, Ashokrao Mane Institute of Pharmacy, Ambap. He has published 15 research and 5 review articles in reputed journals and 2 book chapters. He has 1 year at diploma level, 4 years at UG teaching experience. He is affiliated with professional bodies like MSPC.

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Mr. Pravin S. Patil, has completed MPharm. from Govt. College of Pharmacy, Karad in Biopharmaceutics. He has qualified GATE in 2003 and 2004. Presently he is perusing PhD. in Shivaji University, Kolhapur. Presently, he is working as Assistant Professor and PG Head, Ashokrao Mane College of Pharmacy, Peth Vadgaon. He has published 10 research and 3 review articles in reputed journals. He has 2 years at diploma level, 11 years at UG and PG teaching experience. He is affiliated with professional bodies like MSPC, APTI and KPSF.

Dr. D. A. Bhagwat, has completed his B. Pharm. in 2006 from BVCP, Kolhapur, M. Pharm. in Industrial Pharmacy from S. N. Institute of Pharmacy, Pusad in 2008 and Ph.D. from JJT, University, Rajasthan in 2014. He has total 11 years of Teaching Experience. Currently he is working as Assistant Professor Dept. of Pharmaceutics, Bharati Vidyapeeth College of Pharmacy, Kolhapur. He has authored Six books and published 42 research papers in national and international journals and presented more than 60 papers in various national and international conferences. He is life member of IPA and APTI. He has delivered many lectures in Patient Counseling Course conducted by MSPC, Mumbai.

Dr. Shitalkumar S. Patil, M. Pharm. PhD., MBA presently working as Principal, Ashokrao Mane College of Pharmacy, Peth Vadgaon. He has total 29 years of teaching experience. He has authored 5 books and published more than 75 research article in national and international journals. He is life member of APTI. He is a member of Board of Studies, Shivaji University, Kolhapur.

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Formulation and Evaluation of Herbal Scrub Gel

Pawar Dhanashri N., Pawar Arti P., Dalvi Yogita V.*
Assistant Professor, YSPM YTC Faculty of Pharmacy, Satara, (MH) India-415004

*Corresponding Author E-mail: yogitav.dalvi@gmail.com

Online published on 18 April, 2020.

Abstract

Most of the marketed cosmetics when applied on the skin cause dryness of skin after its long term use which results less life of skin problems of acne and redness. Solution for this problem is use of scrub gel once or twice in week which consist all herbal ingredients which increases cleansing, softening, moisturizing, fairness of skin. In the present work we have formulated the herbal facial scrub by using a different herbal powders and it was evaluated by using the parameters like smoothness, appearance, spreadibility, irritation etc.

Top

Keywords

Scrub gel, softening, and cleansing, moisturizing, fairness.

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Formulation and Evaluation of Polyherbal Soap

Pawar Arti P.[1], Pawar Dhanashri N.[1], Dalvi Yogita V.[2],*
[1]YSPM YTC, Faculty of Pharmacy, Satara, (MH) India-415 004

[2]Assistant Professor,YSPM YTC, Faculty of Pharmacy, Satara, (MH) India-415 004

*Corresponding Author E-mail: yogitav.dalvi@gmail.com

Online published on 18 April, 2020.

Abstract

Polyherbal soap was prepared by using sandal wood and Orange peel extract and evaluated by using various evaluation parameters such as organoleptic characteristics, pH, foam height and retention, skin irritation and high temperature stability. Prepared Polyherbal soap having good appearance better cleansing and foaming effect and doesn't have any side effects.

Top

Keywords

Polyherbal soap, cleansing, foaming.

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ORIGINAL ARTICLE

Synthesis and *in vitro* antimycobacterial potential of novel hydrazones of eugenol

Sachin H. Rohane [a,*], Ashlesha J. Chauhan [b], Neeraj Kumar Fuloria [c], Shivkanya Fuloria [c]

[a] *Department of Pharmaceutical Chemistry, Kadi Sarva Vishwavidyalaya, Gandhinagar 382015, India*
[b] *Department of Chemistry, K.B. Institute of Pharmaceutical Education and Research, Gandhinagar 382023, Gujarat, India*
[c] *Faculty of Pharmacy, AIMST University, Semeling, Bedong, Kedah 08100, Malaysia*

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KEYWORDS

Hydrazone;
Molecular docking;
Antimycobacterial activity

Abstract Fifty one hydrazone derivatives of eugenol were designed and docked with 2NSD and 2X22 (enzymes of H37Rv strain) using Schrodinger v7.4. The selective ten hydrazone derivatives (4, 5, 11, 18, 30, 34, 35, 37, 42, and 45) of eugenol were synthesized via esterification, hydrazination and treatment with different aldehydes. Synthesized compounds were characterized by IR, ^{1}H NMR, and LCMS data. The compounds were evaluated for their antitubercular potential against H37Rv using microplate alamar blue assay (MABA). The study revealed that all synthesized compounds were significantly active at concentration 50 and 100 μg/ml, whereas compound 11 exhibited activity at 25 μg/ml. Present study showed that antitubercular activity of novel hydrazone derivatives of eugenol is strongly connected with the position of the substituent on aromatic aldehyde or ketones.

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1. Introduction

Current decade noticed tuberculosis as the most common infectious disease in the world that being a leading cause of mortality. Regardless of availability of tuberculosis treatment, yet every year 9 million new cases are reported in those 1.5 million are fatal cases (Pitucha et al., 2019). Tuberculosis (TB) is caused by mycobacterium of the "tuberculosis complex", including primarily *Mycobacterium tuberculosis*, *Mycobacterium bovis* and *Mycobacterium africanum* (Sensi and Grass, 1996). Drug discovery and development are complicated, time intense and costly method (Pieczonka et al., 2013). It becomes more expensive when safety, efficacy and other issues are raised. In silicon approach of drug design plays a significant role in all stages of drug development from the initial lead design to final stage of clinical aspect of drug (Abdel-Wahab et al., 2011). Reports suggest eugenol and hydrazones to possess significant anti-tubercular potential (de Almeida et al., 2019; More et al., 2018; Krátký et al., 2017). The chemistry of hydrazones always attract the investigators, as incorporation of these moieties in medicinal compounds due to their biological potential. The hydrazones are known to exhibit wide

* Corresponding author.
E-mail address: sachinrohane29@gmail.com (S.H. Rohane).
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variety of biological activities. They are used as antibacterial agent, anti-tubercular agent, analgesic, anti-inflammatory agent, antiviral agent, antifungal agent, muscle relaxants and antihistamines etc (Yatcheria et al., 2015; Saidugari et al., 2017; Raja et al., 2010; Zheng et al., 2009). In hydrazone, the nitrogen is attached to hydrogen and these hydrazones are stable enough for isolation (March, 1992). However, in some cases, especially with simple alkyl group, they rapidly decompose or polymerizes unless there is at least one aryl group on nitrogen or the carbon (Fuloria et al., 2008). When there is an aryl group the compound are quite stable and these compound are called as Schiff bases. The Schiff reaction is straight forward and proceeds in high yield (Fuloria et al., 2008). Enticed by research evidences, it was thought worthwhile to synthesize some novel hydrazone derivatives of eugenol. We have made an attempt to convert aryloxy moiety into some novel hydrazones via hydrazide and ester intermediates to explore its biological potential. These hydrazones were subjected to molecular docking, synthesis, characterization and antimycobacterial screenings against Mycobacterium tuberculosis.

2. Materials and methods

2.1. General

All chemicals, reagents and solvents were procured from Sigma-Aldrich and Merck Pvt Ltd. and were used without further purification. The reactions were carried out in oven-dried glassware (120 °C) under atmospheric condition. Chemicals and related solvents were procured from Merck Chemicals. From fifty-one in silico docked compounds, the selective ten compounds were subjected to synthesis. The reactions and purity of compounds were monitored by thin layer chromatography (TLC) over percolated plates coated with 0.2 mm Merck 60 F254 silica gel using butanol, acetic acid and methanol (4:3:1, v/v) eluent mixture, and were visualized by UV irradiation (254 nm). The synthesized compounds were purified using column chromatography. The melting points were determined using B-540 melting point apparatus using open capillaries and are uncorrected. The infrared spectra were recorded on a Shimadzu, MIRacle-10, IR Affinity-1 in the range of 400–4000 cm^{-1}. The ^1H NMR spectra were recorded in $CDCl_3$ using Agilent VNMRS 400 instrument at 300 MHz with chemical shift 0–10. The chemical shifts, δ are reported in ppm from 0 to 10 using tetramethylsilane (TMS) as internal standard. The mass spectra were performed using LCMS6103 at m/z values: 0–500.

2.2. Molecular docking

Fifty one compounds (Table 1) were docked in Small-Molecule Drug Discovery Suite of Schrodinger. All compounds were targeted on two enzymes such as 2NSD and 2X22 involved in tuberculosis activity. InhA, the enoyl-ACP reductase in Mycobacterium tuberculosis is an attractive target for the development of novel drugs against tuberculosis. The generated lower energy conformers of all ligands were docked into generated grid of active site of enzymes by XP precision of docking inside Glide-v7.4 (Joshi et al., 2016; Rohane and Makwana, 2019).

2.3. Synthesis of ethyl aryloxy acetate **2**

A mixture of compound 1 (eugenol: 0.1 mol), ethyl chloro acetate (0.1 mol) and anhydrous potassium carbonate (0.15 mol) in dried acetone was refluxed for 12 h. Resultant mixture was distilled off and poured on to ice-cold water and stirred. Residue was extracted with ether and the extract was dried over anhydrous sodium sulphate and was purified under reduced pressure to yield compound 2.

2.4. Synthesis of ethylaryloxyacetyl hydrazine **3**

A mixture of compound 2 (0.05 mol) and hydrazine hydrate (0.075 mol) in ethanol was refluxed for 4 h and after distilling off the solvent the residue was recrystallized from methanol to yield compound 3.

2.5. Synthesis of hydrazones **4, 5, 11, 18, 30, 34, 35, 37, 42,** and **45**

A mixture of compound 3 (0.01 mol) and 2, 4-dihydroxy benzaldehyde (0.01 mol) was refluxed for 2 h using acetic acid. The crystals formed were washed with ice-cold water, dried and recrystallized from methanol to yield compound 4. Following the same procedure using respective aldehydes / ketones, other compounds 5, 11, 18, 30, 34, 35, 37, 42, and 45 were synthesized Scheme 1.

2.6. Anti-tubercular activity

The synthesized compounds (4, 5, 11, 18, 30, 34, 35, 37, 42, and 45) were evaluated for their anti-tubercular potential against standard strain of H37Rv. The method used was microplate Alamar Blue assay (MABA). The final drug concentrations tested were 100–0.2 μg/ml. Plates were covered and sealed with parafilm and incubated at 37 °C for five days. After addition of Alamar Blue reagent and incubating for 24 h, the results were observed. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth.

Being a non-toxic method, it has several advantages such as thermal stability of the reagent, and good correlation with BACTEC radiometric method.

3. Result and discussion

3.1. Molecular docking

The Insilco study of all fifty-one compounds was performed using Small-Molecule Drug Discovery Suite of Schrödinger. The compound 11exhibited good docking score and predicted interaction with enzymes. The docking result of novel hydrazone revealed that the binding energies were in the range of −6.097 kcal/mol to −10.393 kcal/mol, with the minimum binding energy of −10.393 kcal/mol (Table 2). The molecules were tested for structure analysis by the visualization tool. The entire compounds protein-ligand complex showed H - bond with the active site residue TYR 158 and PHE 149of 2NSD (Fig. 1) and GLY96 and TYR 158 of X22 (Fig. 2).

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Table 1 . List of compounds screened for molecular docking.

Comp. No.	Ar'	Comp. No.	Ar'	Comp. No.	Ar'
1	I, I	2	NO_2, O_2N, NO_2	3	Cl, Cl
4	OH, OH	5	I, I	6	OCH_3, OCH_3
7	NO_2, NO_2	8	Cl, Cl	9	OH, HO
10	NO_2, O_2N	11	NH_2	12	Br, I
13	Br, I	14	Br, I	15	Cl
16	Cl, N	17	Cl, N	18	OH
19	OH, I, I	20	OH, I, Br	21	OH, I, Cl

(*continued on next page*)

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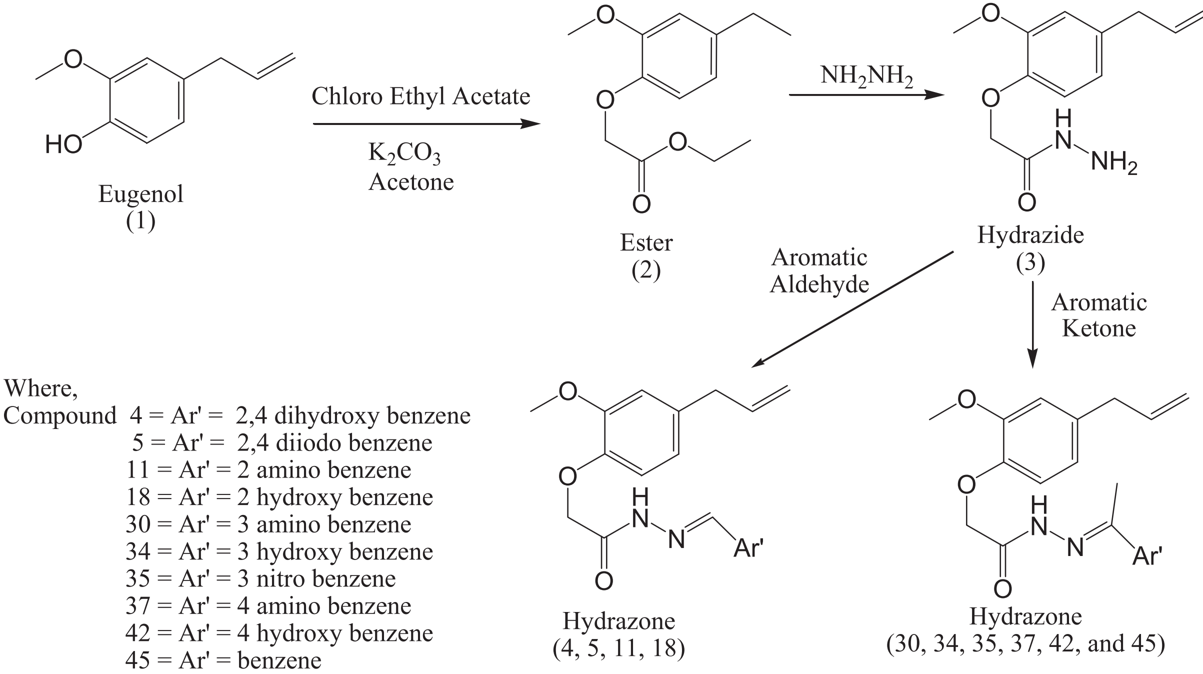
Table 1 (*continued*)

Comp. No.	Ar'	Comp. No.	Ar'	Comp. No.	Ar'
22	I, Cl	23	I, Cl	24	OCH_3
25	NO_2	26	I, I	27	I, I
28	I, OCH_3, I	29	I	30	NH_2
31	Br, I	32	Br, NO_2	33	Br, I
34	OH	35	NO_2	36	I
37	NH_2	38	Br	39	N
40	OH	41	OCH_3, OH	42	OH

Table 1 (*continued*)

Comp. No.	Ar'	Comp. No.	Ar'	Comp. No.	Ar'
43	NO_2	44	I, Br	45	
46		47		48	
49	NO_2	50	Cl	51	OCH_3



Scheme 1 Synthesis of novel hydrazones.

3.2. Synthesis and characterization of synthesized compounds

The ten hydrazone compounds 4, 5, 11, 18, 30, 34, 35, 37, 42 and 45 were selected for synthesis based on their in-silico docking results. The derivatives were synthesized by condensation of arylhydrazide with various aromatic aldehydes or ketones using ethanol. Physical data of all synthesized compounds are given in Table 3 and characterization data mentioned in supplementary file. In the IR spectra, all hydrazone derivatives displayed characteristic band from 1700 to 1650 cm^{-1} attributed to C=O stretching vibration. The N—H stretching vibration of the compounds exhibited a band at near 3150 cm^{-1}. The stretching bands for C=C and C=N groups were observed at 1610–1490 cm^{-1}. In general, the IR stretching frequencies for –OH groups varied for the compounds 4, 18, 34 and 42 in the region 3200–3650 cm^{-1}. In the ^{1}H NMR spectra of all the compounds,

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Table 2 Docking score of compounds.

Title	2NSD			Title	2X22		
	XP GScore	Title	XP GScore		XP GScore	Title	XP GScore
Isoniazid	−3.682	26	−8.15	Isoniazid	−5.451	26	−4.74
1	−8.747	27	−7.37	1	−5.959	27	–
2	−6.847	28	−8.101	2	−4.009	28	−7.486
3	–	29	−6.097	3	−8.007	29	−2.37
4	−10.393	30	−9.021	4	−8.426	30	−7.769
5	−7.919	31	−7.804	5	−6.09	31	−6.092
6	−7.247	32	−6.384	6	–	32	−4.919
7	−7.179	33	−6.955	7	−6.67	33	−6.071
8	−8.387	34	−10.13	8	−5.981	34	−7.448
9	−8.525	35	−9.813	9	−7.538	35	–
10	−7.099	36	−6.538	10	–	36	−6.226
11	−9.5	37	−9.632	11	−7.726	37	−9.092
12	−6.942	38	−9.587	12	−4.965	38	−7.932
13	−8.921	39	−6.818	13	–	39	−5.289
14	−8.216	40	−6.921	14	−8.073	40	−7.674
15	−7.428	41	−6.98	15	−6.721	41	–
16	−7.815	42	−9.747	16	−2.124	42	−8.527
17	−7.266	43	−9.256	17	−6.04	43	−6.551
18	−7.632	44	−8.898	18	−6.625	44	−5.356
19	−7.681	45	−9.049	19	−5.954	45	−6.262
20	−7.466	46	−9.593	20	−4.423	46	−7.758
21	−6.799	47	−9.485	21	−7.037	47	−7.556
22	−7.005	48	−6.469	22	−5.353	48	−4.896
23	−7.867	49	−8.072	23	–	49	−5.725
24	−7.883	50	−6.338	24	−4.742	50	−6.666
25	−8.657	51	−6.175	25	−6.098	51	−4.293

Note-: Sign ‘ – ’ indicates compound does not show any Gscore.

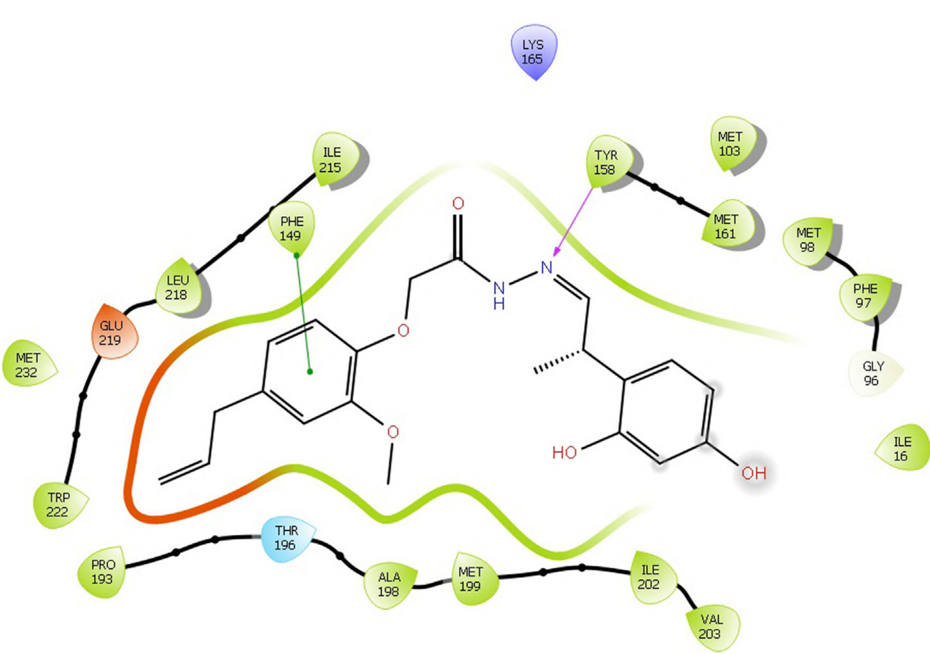

Fig. 1 The Interaction between the compound 4 with the active site of PDB 2NSD.Abbreviation: PDB, protein data bank.

the aromatic and aliphatic protons were observed at the specified ppm scale. Aromatic protons were observed at about δ 6.15–7.78 ppm. Synthesized hydrazones (4, 5, 11, 18, 30, 34, 35, 37, 42 and 45) displayed characteristic NMR signals for —OH, —NH, —CH=N— protons as coupled peaks at δ value of 4.90–5.10 ppm, 7.10–6.90, and 3.35–2.53 respectively.

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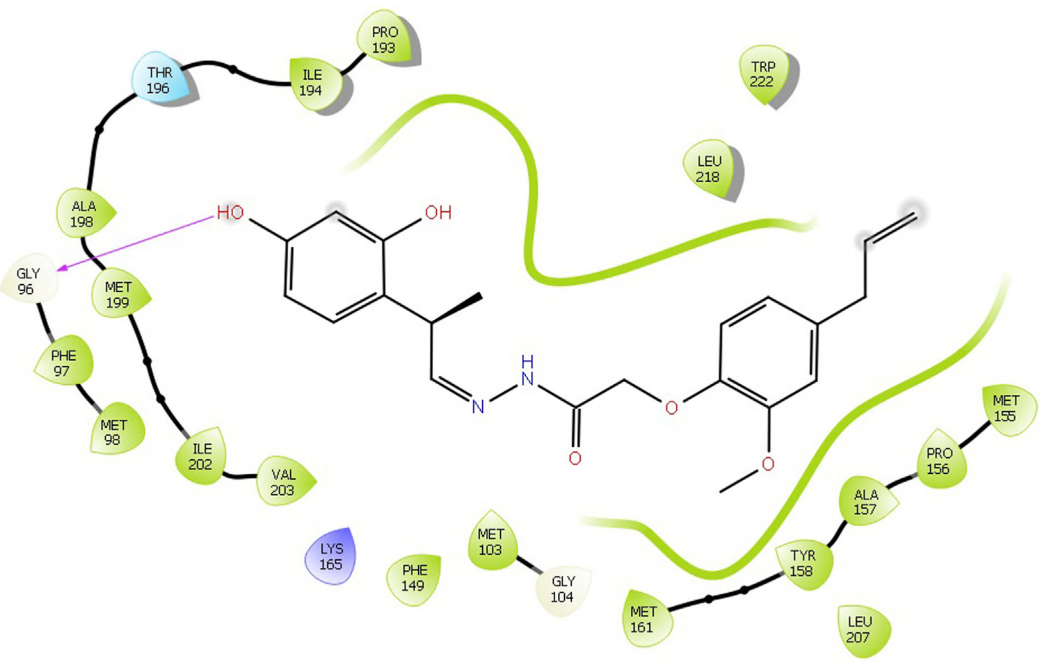

Fig. 2 The Interaction between the compound 4 with the active site of PDB 2X22.

Table 3 Physical characteristics of synthesized hydrazones.

Compound- No	Ar'	Mol. Formula	Mol. Weight	MP °C	Yield %
4		$C_{21}H_{24}N_2O_5$	384.19	220–221	73.08
5		$C_{19}H_{18}I_2N_2O_3$	576.18	235–236	62.00
11		$C_{21}H_{25}N_3O_3$	367.44	191–192	72.03
18		$C_{19}H_{20}N_2O_4$	340.37	229–230	75.00
30		$C_{21}H_{25}N_3O_3$	367.44	190–191	59.15

(*continued on next page*)

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Table 3 (*continued*)

Compound- No	Ar'	Mol. Formula	Mol. Weight	MP °C	Yield %
34	OH	$C_{21}H_{24}N_2O_4$	368.43	217–218	74.08
35	NO_2	$C_{21}H_{23}N_3O_5$	397.42	235–236	60.08
37	NH_2	$C_{21}H_{25}N_3O_3$	367.44	190–191	72.03
42	OH	$C_{21}H_{24}N_2O_4$	368.43	219–220	76.90
45		$C_{21}H_{24}N_2O_3$	352.43	184–185	65.55

Table 4 Antimycobacterial activity of novel hydrazones.

Compound	100 μg/ml	50 μg/ml	25 μg/ml	12.5 μg/ml	6.25 μg/ml	3.12 μg/ml	1.6 μg/ml	0.8 μg/ml
4	S	S	R	R	R	R	R	R
5	S	S	R	R	R	R	R	R
11	S	S	S	R	R	R	R	R
18	S	S	R	R	R	R	R	R
30	S	R	R	R	R	R	R	R
34	S	S	R	R	R	R	R	R
35	S	S	R	R	R	R	R	R
37	S	S	R	R	R	R	R	R
42	S	S	R	R	R	R	R	R
45	S	S	R	R	R	R	R	R
Isoniazid	S	S	S	S	R	R	R	R

NOTE: S - Sensitive R- Resistant.

3.3. Antitubercular activity

The compounds 4, 5, 11, 18, 30, 34, 35, 37, 42 and 45 were evaluated for *in vitro* antimycobacterial activity against *M. tuberculosis* H37Rv by microplate alamar blue assay (MABA) method, using isoniazid as standard. The results expressed in minimum inhibitory concentration (MIC) are given in Table 4 and the colour change observed during assay method is shown in Fig. 3. The obtained results indicate the biological potential and varying activity depending on the type of substituent on

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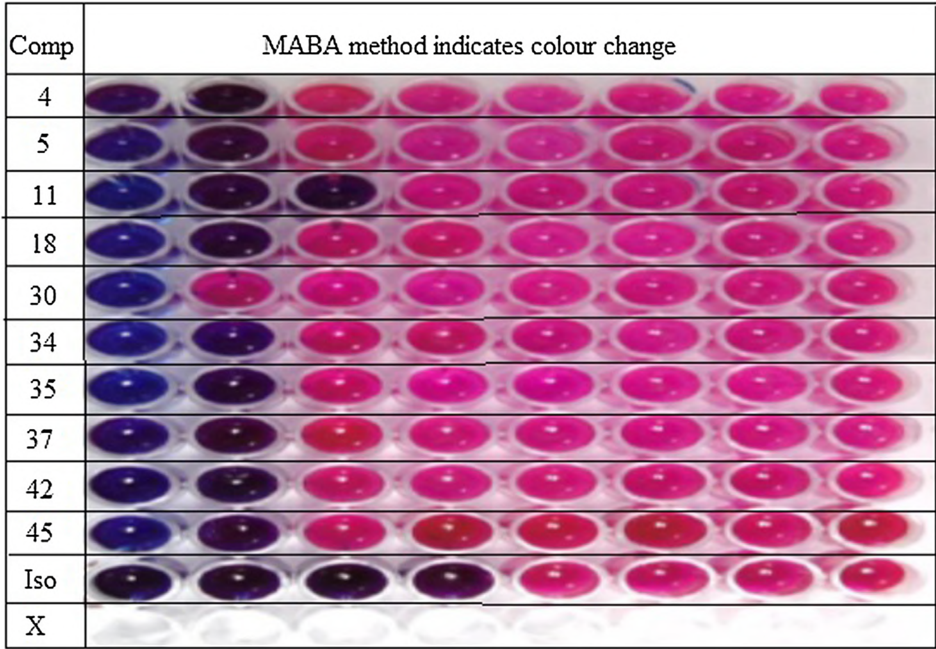

Fig. 3 Assessment of Microplate Alamar blue assay result.

hydrazide nucleus. The results of anti-tubercular activity revealed all newer hydrazones possess inhibitory potential against *Mycobacterium tuberculosis*. Whereas compound 11 possess highest sensitivity against *M. tuberculosis* H37Rv at 25 μg/ml level. The antibacterial activity is strongly connected with the position of the electronegative substituent on phenyl ring of aldehydes in relation to the hydrazone skeleton (Fuloria et al., 2017).

4. Conclusion

The molecular docking studies investigating hydrazone derivatives using the enzyme 2NSD and 2X22 as their potential biological target indicate that the amino, azide, hydroxyl and phenyl nucleus of hydrazone derivatives spacer play an important role in interactions with the active site such as TYR 158, ILE 215, GLU 219, PHE 97 and PHE 149 as the most active amino acid residues. Present study establishes the synthesis of newer hydrazone derivatives 4, 5, 11, 18, 30, 34, 35, 37, 42 and 45 by Schiff's reaction of aryloxy hydrazide with appropriate aldehyde or ketone. The structures of synthesized compounds were confirmed by spectroscopic methods. In the prepared hydrazone all compounds showed significant anti-tubercular action where as compound 11, exhibited highest anti-tubercular activity.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2019.09.004.

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FORMULATION AND EVALUATIONS OF HERBAL LIPSTICK

Drx. Sneha Yadav*, Dr. V. K. Redasani and K. J. Baid

Department of Pharmaceutical and Pharmacongosy Technology, Shivaji University College of Pharmacy, Yspm Ytc's Campus, Satara, India.

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***Corresponding Author**
Drx. Sneha Yadav
Department of Pharmaceutical and Pharmacongosy Technology, Shivaji University college of pharmacy, Yspm Ytc's Campus, Satara, India.

ABSTRACT

They because it has become part of our society and fashion, cosmetics areextremely in demand since historical times till the day. Lipsticks are most commonly used to enhance lip appeal, and they also aid greatly in preserving health and happiness. For this intention and objective, an attempt was made to frame herbal lipsticks using colored natural, and the lipsticks were tested on their sensory examination, such as spreading, stiffness, shine and gloss, in order to achieve a good product. Such lipstick preparations contain natural ingredients such as carnauba wax, white bees wax oil of peppermint, oil of castor, oil of almond. Because of various adverse effects of the available synthetic preparation, the present work was designed to formulate a herbal lipstick with minimal to no side effects that will be used extensively by our society's women with great compensation and satisfaction.

KEYWORDS: Red Barrie, Blue Barrie, Herbal Lipstick, Cosmetics.

1. INTRODUCTION

Cosmetics include skin care creams, lotions, powders, eye and facial make-up, permanent waves, colored contact lenses, hair colors, hair sprays, and gels, deodorants, infant products, bath oils, bubble baths, bath salts, butters, and many other types of cosmetics in both developing and developed countries.[1,2]

The world herbal is a symbol of safety in contrast to the synthetic one which has adverse effects on human health. Herbal preparations via ; herbal tablets , herbal tonics, herbal paste, herbal shampoo, herbal tonic, herbal paste, herbal sindhur, herbal contraceptives and herbal lipstick has become popular among the consumer herbal medicine represent the fastest

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growing segment to heal the various aliment possibly, the herbal users desire to assume control over health.[3,4]

The use of the drug has increased in the present days and a lot of improvements occur in the choice of stain texture colors, lipstick luster.[5] It can be learned from the evidence that lipstick is being sold in hundreds of shades of colors to satisfy women's demand.[6] Now it's time to stick to the use of herbal products and embrace a more natural way of life in the whole world for days.[6]

Ideal characteristics of good lipstick

- It should be non- irritant.
- It should have required plasticity.
- It should nontoxic.
- That should be physically and chemically stable.
- It is not expected to dry up on board.
- It should be heavily particulate free.
- After application it can retain lip color for longer periods.
- This should offer sweating-free shiny and smooth appearance.
- It should look, smell and feel good.

It should not fuse or harden within acceptable climatic temperature variation.[7]

2. Noxious lipstick issues

while lipstick has a colorful background and a specific market, it also suffers from some dangerous disadvantage. Recent research has found that the lipstick contains traces of lead and other heavy metals such as antimony, arsenic, cadmium that cause serious health problems and can be carcinogenic or even fatal in extreme form as it is based on human ingestion.[9] Such lip care products cause some allergic reactions, the most severe being allergic touch cheilitis of the vermilion margin of the lip often spreading to the adjacent perioral area that may be acute or chronic.[10]

2.1 Lead traces

A recent federal study showed that 400 shades of common lipstick contained trace amounts of lead which intensified a continuing dispute between regulators and consumer activists about how much lead is safe in cosmetics.[11] According to tests by the Food and Drug Administration, five lipsticks manufactured by L'Oreal and Maybelline, operated by L'Oreal

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USA, rated among the top 10 most contaminated of cosmetics.[10] Two Cover Girl and two NARS lipsticks have landed in the top 10, much as Stargazer did one. In October 2007, a study by the US consumer group Campaign for Healthy Cosmetics found that 60 per cent of the lipsticks tested contained trace amounts of lead, particularly in red lipsticks. Lead rates ranged from 0.03 parts to 0.65 parts per million. One third of lead-containing lipsticks reached the mark of 0.1ppm limit set by the U.S food and drug administration for lead in candy.[12]

2.2 Description of fruit

Red barrie: (Rubus idaeus)[12,13,14]

Botanical name	Red raspberry, Rubus Idaeus
Taxonomy	The raspberry, bramble fruit of genus rubus belong to family Rosaceae.
Geographical area	The wild beery gathering remains a popular activity Europe and north American
Chemical constituent	Anthocyanin, flavonoids, stilbenoids, phenolic acid, tannin and lignans Ellagic acid, cyanidins, pelargonidins Folate, omega 3 fatty acid.
Medicinal uses	Antioxidant and anti- inflammatory. Obesity and Blood sugar benefits. Anti-cancer

- **Blue Barrie: (Rubus ulmifolius)[15,16]**

Taxonomy	The blueberry fruit of genus belong to vaccinium and cyanococcus belong to family Ericaceae. n subfamily vaccinoideae genus vaccinium
Geographical area	· North America and Europe region · Atlantic Canada · Northeastern united states
Chemical constituent	· Anthocyanins, anthocyanidine (phenolic aglycone) · Chlorogenic acid, flavonoids, alphalinolenic acid and vitamins.
Medicinal uses	· Maintaining healthy bones · Skin health · Lowering blood pressure · Managing diabetes

3. MATERIAL AND METHOD

3.1 Material

Sr.no	Material	Manufactured by
1	Red Raspberry (Red Barriers)	Magsa Moulde, Food Market
2	Rubus Ulmifolius (Blue Berry)	Magsa Moulde, Food Market
3	Bees Wax	Vedant Lab
4	Carnuba wax	Vedant Lab

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5	Lanolin	Vedant Lab
6	Mentha Piperita (Peppermint Oil)	Dhanvantari Ayurveda
7	Prunus amygdalus Oil (Almond Oil)	Dhanvantari ayurved
8	Orange Essence	Dhanvantari ayurved
9	Vanilla Essence	Dhanvantari ayurved

3. 2 Method

Herbal lipstick formulation requires the basic manufacturing process such as,[17]

- **Pigment premilling**

The first step involved in the formulation of herbal lipstick is pigment premilling where the agglomerates in the powder are broken down to give the lipstick a homogeneous smoothness and even colour.

- **Melting and mixing**

The next step involved is the melting and mixing stage, since waxes are solid at room temperature and can not be combined with other ingredients to make the waxes melted simple to make this process. Typically it can be combined with oil, and the pigment and other additives are added and blended to form a homogeneous substance to the melted foundation.

- **Molding**

Molding is the actual phase in which the molten lipstick is poured into metal or plastic mold, the mixture is poured when it is hot so it is helpful to harden and then removed with a slight pressure from the mole.

- **Flaming**

Flaming is the last stage in which the lipstick passes through the flame, is usually held and twisted in the flame for up to a second and then removed to prevent melting and losing shape to achieve a shiny finish and then put in the bottle. Different formulations are made from Test 1 to Test 5 to find the superior lipstick with colorant and oil as variable parameter.

Basic ingredient required

Sr. no.	Ingredients	% (w/w)
1	Bees wax	10
2	Caruba wax	10
3	Lanolin	15
4	Mentha Piperita (Peppermint Oil)	65
5	Prunus amygdalus Oil (Almond Oil)	65
6	Orange Essence	Adequate
7	Vanila Essence	Adequate

A cosmetic lip care should have the following criteria from a customer perspective: attractive color Homogeneous color when applied

- Attractive colour
- Homogeneous colour when applied
- Pleasant smell and taste
- Easy to apply
- No staining or bleeding into fine line surrounding the lips
- Long lasting effect

3.3. Evaluations test

The quality control parameter used in efficient are

- Melting point
- PH
- Surface anomalies
- Solubility test
- Skin irritation test
- Color
- Microbiological test

1. Melting point

Apparatus: Flat Bottom Tube, Thermometer

Procedure: Place the lipstick in a flat bottom tube with protruded salve. Fix the thermometer through a cork, so that the thermometer bulb just touches the salve of the lipstick. Place this arrangement into a 1-liter beaker filled with water over the upper tip of the lipstick salve to a point one centimetre above. Place this arrangement into a 1-liter beaker filled with water over the upper tip of the lipstick salve to a point one centimetre above. Warm the water gradually while stirring so that temperature increases at a rate of no more than 2 ° C per minute. As the temperature increases to about 45 ° C, increase the temperature to 1 ° C per minute. Keep the lipstick salve continuously. Report when the temperature is in when the salve begins to bend and lose form.[18]

2. PH test

The pH of herbal lipstick formulated was determined using pH paper[19]

3. Surface anomalies

This is being examined to identify any surface defects, such as surface crystal formation, mold contamination, fungi etc.[20]

4. Solubility test

The formulated herbal lipstick was dissolved to assess the solubility in various solvents.[21]

5. Skin irritation test

The drug is spread over the skin for 10 min.[22]

4. RESULT AND OBSERVATION

There has been enormous increase in women's use of cosmetics over the last few decades. The hazards caused by these chemicals, however, have recently come to the forefront. The goal of the present research formulating and evaluating herbal lipsticks was to formulate a lipstick using herbal ingredients with the hope of minimizing the side effects caused by the synthetic ones available. Therefore, from the present investigation it has been concluded that this formulated herbal lipstick has a better choice for women with limited side effects although a thorough clinical trial can be performed for better efficacy to access the formulation.

- **Melting point**

The melting point of the formulated herbal lipsticks was evaluated and the result indicates that formulation 6 has highest melting point compared to other formulations.

- **PH**

The pH of the formulated herbal lipstick was evaluated as quality control test and as a result it was found that four formulations have PH range of 6.

- **Microbiological test**

Any product will be in jeopardy by the growth of micro-organism hence it is essential to determine the number of microorganism that has grown on the product through microbiological test. This quality control test has been done on all six formulations and it has been found that formulation 6 is less susceptible to the growth of microorganism.

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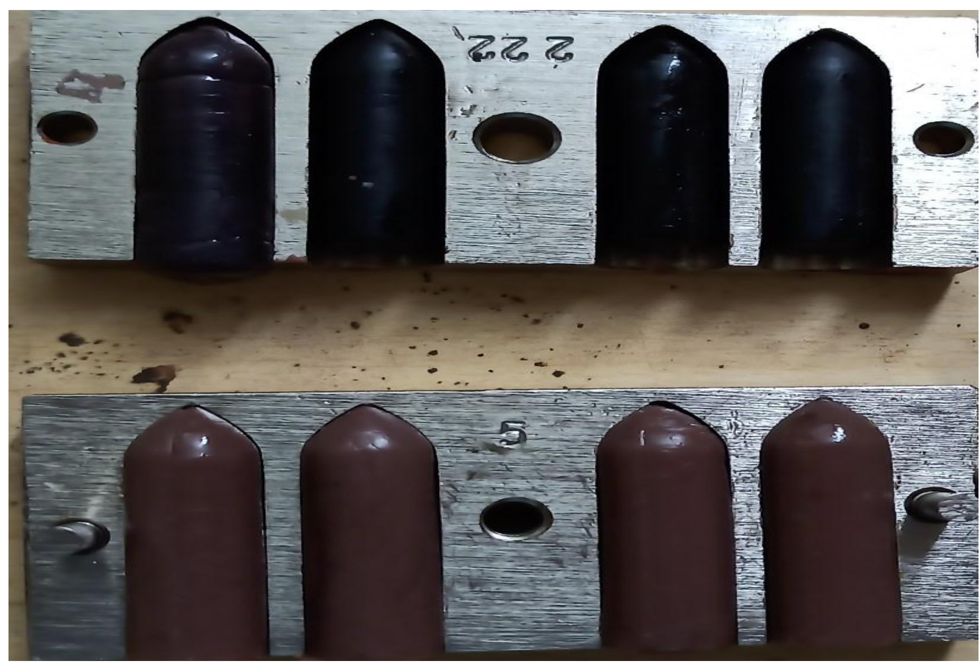

Figure 1: Formulation of herbal Lipstick.

Figure 2: Formulation herbal lipstick in container.

1. **Red barrie: Rubus idaeus**

Table no. 1: Rubus idaeus parameter

+ = Good, ++ = Best, +++ =Excellent

Parameter	Trial 1	Trial 2	Trial 3	Trial 4
Colour	White	Pale Pink	Deep Red	Pinkish Red
Surface of Anomalies	No Defects	No Defects	No Defects	No Defects
Aging Stability	Rough	Smooth	Smooth	Smooth
Perfume Stability	+	++	++	+++
Solubility Test	$CHCl_3$	$CHCl_3$	$CHCl_3$	$CHCl_3$
Skin Irritation Test	No	No	No	No

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2. Blue barrie: Rubus ulmifolius

Table no 2: Rubus ulmifolius parameter.

+ = Good, ++ = Best, +++ =Excellent

Parameter	Trial 1	Trial 2	Trial 3	Trial 4
Colour	White	Pale purple	Deep Purple	Reddish Purple
Surface of Anomalies	No Defects	No Defects	No Defects	No Defects
Aging Stability	Rough	Smooth	Smooth	Smooth
Perfume Stability	++	++	+++	+++
Solubility Test	$CHCl_3$	$CHCl_3$	$CHCl_3$	$CHCl_3$
Skin Irritation Test	No	No	No	No

5. CONCLUSION

Study concluded that herbal lipstick can be successfully formulated using different natural ingredients such as white bees wax, butter , peppermint oil, almond oil, Vanilla & rose essence, blue berry extract , red berry extract powder, will be better option for synthetic colouring agents which may arise different side effects. Consumers can take safe and effective advantage of this herbal lipstick after thorough clinical trials.

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Criterion III: - Research, Innovations and Extension

Sr. No.	Title of paper	Name of the Author/s	Name of Journal	Link to article / paper / abstract of the article
1	Freeze dried multicomponent inclusion complexes of quercetin: Physicochemical evaluation and pharmacodynamics study	AS kulkarni R.J.Dias VS Ghorpade	Journal of research in pharmacy	file:///C:/Users/Admin/Downloads/MPJ_679.pdf
2	Estimation of heavy metals from shankhavati tablet	S.S.Dhebe A M Bhagwat SS Deshpande SV Garad	World Journal of pharmaceutical research	https://wjpr.s3.ap-south-1.amazonaws.com/article_issue/1553130327.pdf
3	An Empirical study on the employability skill of pharmacy under graduates in satara region	P R bhosale A.M.Bhagwat S.H.Rohane	European Journal of pharmaceutical and medical research	file:///C:/Users/Admin/Downloads/Publishedarticle_1554011386ejpmr2019-6-4322-329.pdf
4	Importance of force decredation study in pharmaceutical industry-A Review	S.R chavan A M Bhagwat Mahesh Rao A.P.Choudhari	World Journal of pharmaceutical research	NA
5	Study of fructose-glucose ratio in different samples of honey available in satara region	S R Ghadge A M Bhagwat SS deshpande SK budhavale	World journal of pharmacy and pharmaceutical sceince	https://www.researchgate.net/profile/Avinash-Bhagwat-2/publication/370471000_STUDY_OF_FRUCTOSE-GLUCOSE_RATIO_IN_DIFFERENT_SAMPLES_OF_HONEY_AVAILABLE_IN_SATA

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6	Formulation and Evaluation of Polyherbal Soap	Arti P. Pawar, Dhanashri N. Pawar1, Yogita V. Dalvi	Research Journal of Topical and Cosmetic Sciences;	https://rjtcsonline.com/HTMLPaper.aspx?Journal=Research%20Journal%20of%20Topical%20and%20Cosmetic%20Sciences;PID=2019-10-1-6
7	Delaying effect of polyherbal formulation on cataract in stz-in ic induced diabetic wistar rat	K K mali S S ligade R.J.Dias	Indian journal of pharmaceutical sceiences	https://www.ijpsonline.com/articles/delaying-effect-of-polyherbal-formulation-on-cataract-in-stznicinduced-diabetic-wistar-rats.pdf
8	Home Automation Using Arduino And Iot	Pratiksha R. Patil Chaitali Ghadge	International Journal for Science and Advance Research In Technology	https://ijsart.com/Home/IssueDetail?id=30030
9	In Silicostudy for prediction of multiple pharmacological activities of novel hydrazine derivatives	S H Rohane Ashlesha makwana	Indian Journal of chemistry	https://nopr.niscpr.res.in/handle/123456789/45930
10	Extraction, Characterization and functionalization of tamarind gum	K K mali S S ligade R.J.Dias	Research journal of pharmacy and technology	https://www.researchgate.net/profile/Kailas-Mali/publication/333055878_Extraction_Characterizatio

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				n_and_Functionalization_of_Tamarind_Gum/links/5d08f349458515ea1a6f0fcb/Extraction-Characterization-and-Functionalization-of-Tamarind-Gum.pdf
11	Pharmacognostic investigation of lanata camera leave	S D patil D M Nirmale	International Journal of pharmacognocy	NA
12	Health related quality of life questioner of COPD patient	S D patil D M Nirmale	Journal of Hospital Pharmacy	NA
13	Traditional aproches for the treatment of diabetes	Aryan Langeh PV bhokare Ashish Thorat	European Journal of biomedical and pharmaceutical sceince	NA
14	Formulation, design and characterization of mucoadhesive buccal film of nebivolol using factorial design	Anilkumar J. Shinde , Deepti S. Waghmare, Rahul S. Dalvi and Harinath N. More	International Journal of Pharmaceutical sciences and Research	file:///C:/Users/Admin/Downloads/8-Vol.-9-Issue-5-May-2018-IJPSR-RA-8934.pdf
15	Utilization of Press mud for Improvement of Strength of Interlocking Bricks	Mr. Shaikh. A. N. , Miss.Deshmukh P.S. , Mr. Shelar V.E.	International Journal of Scientific Research in Engineering and Management	https://dnyanshree.edu.in/NAAC/Criterian-III/3.3.1/DIET%20CRIT%203_3.3.1_49_SAN_C.pdf

Freeze dried multicomponent inclusion complexes of quercetin: Physicochemical evaluation and pharmacodynamic study

Anita KULKARNI [1], **Remeth DIAS** [2]*, **Vishwajeet GHORPADE** [3]

[1] Department of Pharmaceutical Chemistry, Government College of Pharmacy, Karad, Maharashtra, India
[2] Department of Pharmacy, Government Polytechnic, Jalgaon, Maharashtra, India.
[3] Department of Pharmaceutics, YSPM's Yashoda Technical Campus, Wadhephata, Satara-415011, Maharashtra, India.
* Corresponding Author. E-mail: rjdias75@rediffmail.com (R.D.).

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ABSTRACT: The present study was undertaken to investigate the effect of cyclodextrin (CD) complexation in presence of hydrophilic polymer on physicochemical properties as well as anti- inflammatory activity of quercetin (QUN). The initial phase solubility studies were carried out in presence and absence of hydrophilic polymers (hydroxypropylmethylcellulose, polyvinylpyrrolidone K30 and poloxamer 188) to study their effect on the stability and complexation efficiency of CDs. The binary (QUN-βCD and QUN-HPβCD) and multicomponent (QUN-βCD-POLO and QUN-HPβCD-POLO) inclusion complexes of QUN prepared using lyophilization technique. The complexes were subjected to DSC, ATR-FTIR, XRPD and SEM analysis, and evaluated for drug content and saturation solubility. The phase solubility studies revealed the formation of QUN-βCD and QUN-HPβCD complexes with 1:1 stoichiometry. The incorporation of POLO increased the stability as well as complexation efficiency of CDs. FTIR and DSC analysis indicated hydrogen bonding interaction POLO with QUN and CDs. XRD and SEM analysis revealed greater amorphization in case of multicomponent inclusion complexes. All complexes showed uniform drug content. QUN-HPβCD-POLO exhibited maximum solubility than the physical mixtures and other complexes. The pure QUN and QUN-HPβCD-POLO were tested for in *vivo* anti-inflammatory activity by Carrageenan induced rat paw edema method. QUN-HPβCD-POLO exhibited significant increase in anti-inflammatory activity as compared to pure QUN.

KEYWORDS: Quercetin; β-cyclodextrin; hydroxypropyl-β-cyclodextrin; lyophilization; ternary complex.

1. INTRODUCTION

Quercetin (QUN) is a bioflavonoid (3,5,7,3′,4′-pentahydroxyflavone) widely distributed in many plants (see Figure 1). It is also a frequent component of major dietary constituents such as onions, apples, red wine and green tea. Flavonoids, intensely coloured polyphenolic phytochemicals, exhibit various biological activities. Nowadays research is being focused on the antioxidant, antitumour, antiinflammatory and antibacterial activity of flavonoids. In literature wide range of biological properties of QUN are reported which are often related to its antioxidant activity. Besides, it is also known for its anti-inflammatory, antihypertensive, vasodilator effects, antiobesity, antihypercholesterolemic and antiatherosclerotic activities [1–4]. QUN shows a strong inhibitory effect on the growth of several human and animal cancer cell lines and enhances the antiproliferative effect of cisplatin both *in vitro* and *in vivo*. However, the oral bioavailability of QUN is found to be less than 17% in rats and approximately 1% in humans which may be due to its low aqueous solubility [5–7].

From past few years, technique of cyclodextrin inclusion complexation is being widely used to improve the physicochemical properties of drugs such as solubility, chemical stability and bioavailability [5,8]. Cyclodextrins (CDs) are cyclic oligosaccharides consisting (α-1, 4)-linked α-D-glucopyranose units containing a relatively hydrophobic central cavity and hydrophilic outer surface [9]. In an aqueous environment, cyclodextrins form inclusion complexes with many lipophilic drug molecules through a process in which water molecules located inside the central cavity are replaced by either the whole drug molecule or more

frequently, by some lipophilic structure of the molecule. The natural cyclodextrins, in particular β-cyclodextrin (βCD), have limited aqueous solubility and their complex formation with

Figure 1. Chemical structure of QUN.

lipophilic compounds frequently results in precipitation of solid cyclodextrin complexes [10]. The application of βCD in the pharmaceutical formulations is limited owing to its low aqueous solubility [5].

2-hydroxypropyl-β-cyclodextrin (HPβCD); a hydroxyalkyl β-bcyclodextrin derivative has been successfully employed in pharmaceutical formulations due to its amorphous nature and enhanced water solubility than βCD [11]. However, the amount of HPβCD to be used in the pharmaceutical formulations is limited due to its toxicity [12]. The problems associated with βCD and HPβCD can be overcome by addition of small amounts of auxiliary substances such as hydrophilic polymers [13], hydroxyl acids [14] and/or amino acids [8] to the complexation media. This results in the formation of multicomponent inclusion complexes of CDs, where the auxiliary substances improve the complexation efficiency of CDs [11].

There are some reports where inclusion complexes of QUN with βCD and HPβCD have been prepared to enhance its solubility using freeze drying [5] and spray drying techniques [1]. Besides, the effect of complexation of QUN with three kinds of CDs in solution state on the antioxidant activity of QUN has been studied by Jullian and coworkers [3]. The complexes of QUN with βCD, HPβCD and sulfobutyl-βCD in solution state were studied in order to investigate the effect of complexation on chemical stability and water solubility of QUN [2]. To our knowledge, no reports related to the effect of multicomponent inclusion complexes of QUN with CDs and hydrophilic polymers on its solubility and anti-inflammatory activity are available.

The aim of this study was to prepare freeze dried multicomponent (ternary) inclusion complexes of QUN with βCD and HPβCD in presence of a suitable hydrophilic polymer in order to minimize the amount of CDs, increase the stability of the formed inclusion complexes and enhance the solubility of QUN so as to maximize its anti-inflammatory activity. Phase solubility studies were performed to study the stoichiometry between QUN and CDs, and to select the hydrophilic polymer that increases the stability of the formed complexes and improves the complexation efficiency of CDs. The prepared complexes were characterized by attenuated total reflectance-fourier transform infrared spectroscopy (ATR-FTIR), differential scanning calorimetry (DSC), scanning electron microscopy (SEM) and X-ray powder diffractometry (XRPD). The complexes were evaluated for saturation solubility and drug content. The in *vivo* anti-inflammatory activity of the complex exhibiting high solubility was evaluated using Carrageenan induced rat paw edema method.

2. RESULTS AND DISCUSSION

2.1. Phase solubility studies and interaction of QUN with CDs

The phase solubility diagram of QUN in aqueous solutions of βCD and HPβCD in the presence or absence of hydrophilic polymers (HPMC, PVP and POLO) are shown in Figure 2 (a and b). These diagrams exhibited a linear relationship between the amount of solubilized QUN and concentration of solution of CDs (A_L-type of curves) which signifies formation of water soluble complexes [15]. Table 1 shows the values of slope, stability constant (Kc) and complexation efficiency of the systems considered under phase solubility studies. The slope of phase solubility curves for all the systems was found to be less than one indicating 1:1 stoichiometry between QUN and CDs [16]. The presence of hydrophilic polymer caused increase in the stability of the formed complexes along with enhancement in the complexation efficiency of the CDs. This can be attributed to the molecular interactions in between QUN, CDs and hydrophilic polymers such as hydrogen bonding, formation of hydrophobic bonds and van der Waals interaction [17–19].

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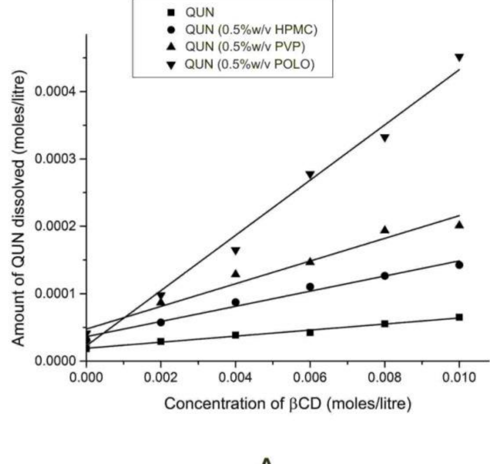

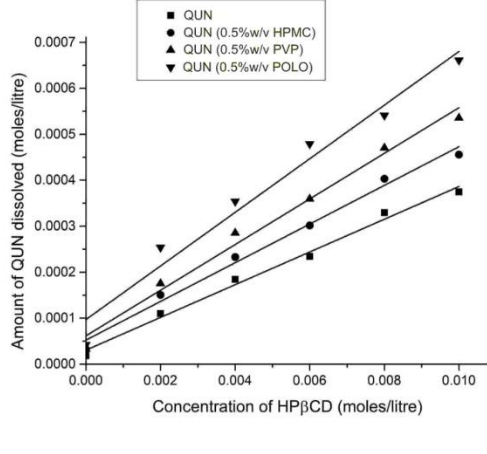

Figure 2. Phase solubility profile of QUN-βCD (A) and QUN-HPβCD (B) systems in distilled water (■) and in presence of 0.5 %w/v HPMC (●), 0.5 %w/v PVP (▲) and 0.5 %w/v POLO (▼).

Table 1. Phase solubility data of binary and ternary inclusion complexes of QUN with βCD and HPβCD.

QUN/CD systems	Phase solubility parameters			
	Slope	**$K_{c,1:1}$ (M^{-1})**	**C.E.**	**S_o (moles/litre)**
QUN/βCD	0.0044	240.29±6.86	0.0045	$1.86×10^{-5}$
QUN/βCD/HPMC (0.5%w/w)	0.0112	597.07±11.98	0.0113	$3.12×10^{-5*}$
QUN/βCD/PVP (0.5%w/w)	0.0168	888.86±8.32	0.0171	$3.32×10^{-5*}$
QUN/βCD/POLO (0.5%w/w)	0.0409	2111.71±14.42	0.0427	$4.13×10^{-5*}$
QUN/HPβCD	0.0355	1842.43±13.16	0.0368	$1.86×10^{-5}$
QUN/HPβCD/HPMC (0.5%w/w)	0.0445	2286.90±10.16	0.0466	$3.12×10^{-5*}$
QUN/HPβCD/PVP (0.5%w/w)	0.0495	2531.76±7.42	0.0521	$3.32×10^{-5*}$
QUN/HPβCD/POLO (0.5%w/w)	0.0634	3193.08±16.81	0.0677	$4.13×10^{-5*}$

$K_{c,1:1}$: stability constant; S_o: intrinsic solubility; QUN: quercetin; βCD: β-cyclodextrin; HPβCD: hydroxypropyl-β-cyclodextrin; HPMC: hydroxypropyl-methylcellulose; PVP: polyvinylpyrrolidone K30; POLO: poloxamer 188. The values of slope C.E. and So are expressed as mean (n=3) whereas value of $K_{c,\ 1:1}$ is expressed as mean ± S.D. (n=3). * indicates solubility of QUN in presence of respective hydrophilic polymers.

Table 2 represents the Gibbs free energy change ($G_{tr}°$) in the systems with increase in the concentration of CDs. A decrease in the $G_{tr}°$ values with respect to increase in the CD concentration indicated the spontaneous nature of QUN solubilization suggesting that the reaction became more favorable with the increase in the concentration of CDs. The negative $G_{tr}°$values indicated the exothermic nature of the complexation process [20]. The system comprised of QUN, HPβCD and POLO showed maximum stability, high complexation efficiency and lowest value of $G_{tr}°$. This may be due to high solubility of HPβCD than βCD and ability of POLO to enhance the solubilizing efficiency of HPβCD by interacting with the functional groups present on the exterior of the HPβCD and QUN [21]. Due to its amphiphilic nature, POLO can also improve the solubility of the free uncomplexed drug [22]. As POLO was found to be more efficient ternary component amongst the selected polymers, it was used for the preparation of the freeze dried multicomponent inclusion complexes of QUN.

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Table 2. Gibbs free energy change (ΔG_tr) in Joules/ mole.

System	Moles of CDs	QUN/βCD system			
		Without polymer	HPMC (0.5%w/v)	PVP (0.5%w/v)	POLO (0.5%w/v)
QUN/βCD	0.002	-1106.59	-2763.41	-3775.98	-4066.73
	0.004	-1787.38	-3788.49	-4734.52	-5344.12
	0.006	-2019.85	-4359.08	-5057.65	-6619.67
	0.008	-2671.12	-4692.82	-5739.18	-7060.87
	0.01	-3065.31	-4988.21	-5829.53	-7812.84
QUN/HPβCD	0.002	-4351.07	-4784.12	-5493.93	-5882.08
	0.004	-5618.43	-6189.87	-6686.10	-7215.45
	0.006	-6204.44	-6823.86	-7253.22	-7956.97
	0.008	-7040.59	-7535.44	-7911.77	-8254.63
	0.01	-7353.55	-7949.54	-8228.41	-8896.33

CDs: cyclodextrins; QUN: quercetin; βCD: β-cyclodextrin; HPβCD: hydroxypropyl-β-cyclodextrin; HPMC: hydroxypropylmethylcellulose; PVP: polyvinylpyrrolidone K30; POLO: poloxamer 188.

2.2. Characterization of the inclusion complexes

2.2.1. DSC analysis

DSC can be used to investigate the interaction between host and guest molecules. When guest molecules are included in CD cavity, their melting points usually shift to a different temperature or disappear [17]. DSC patterns of QUN and all complexes are shown in Figure 3. The thermogram of QUN showed a characteristic endothermic peak at 319.10^{0}C close to its melting point 316.0 indicating presence of crystalline phase. The broad endothermic peak at 126.59^{o}C (T_{onset} = 99.94^{0}C) represents removal of water of crystallization as QUN is a dihydrate molecule [23]. The DSC thermogram of βCD displayed a broad endotherm at 99.95^{o}C, indicating a loss of water due to dehydration process. A melting endotherm was observed at 335.32^{o}C. The thermal curve of pure HPβCD is characterized by a broad endothermic peak at 75.48 ^{o}C, indicating a loss of water due to dehydration process [24].

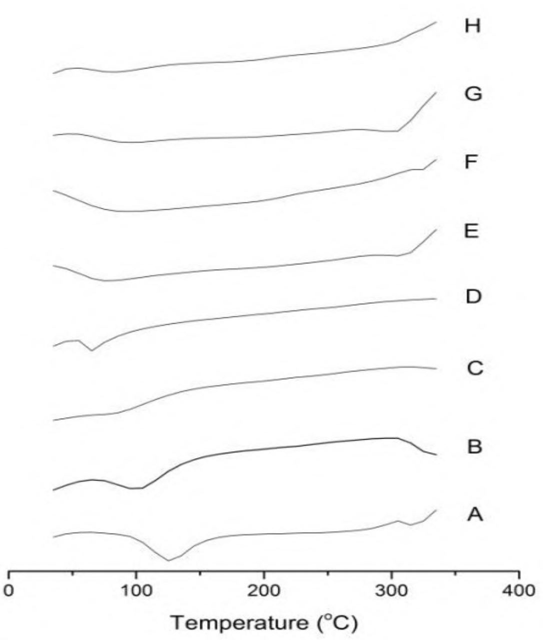

Figure 3. DSC thermograms of QUN (A), βCD (B), HPβCD (C), POLO (D), QUN-βCD (E) and QUN-HPβCD (F) binary complexes, QUN-βCD-POLO (G) and QUN-HPβCD-POLO (H) ternary complexes.

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It showed melting endotherm at 366.62°C corresponding to its melting point. The DSC thermogram of POLO showed a sharp endothermic peak at 60.28°C indicating its crystalline nature. In the thermogram of QUN-βCD binary complex, the melting endotherm of QUN was found to be diffused at 307.19°C, while the thermogram of QUN-HPβCD binary complex showed a small diffused endothermic peak at 322.78°C. In case of QUN-βCD-POLO ternary complex, the characteristic endothermic peak of QUN was diffused and shifted to 299.52°C indicating formation of inclusion complex [25]. The thermogram of QUN-HPβCD-POLO ternary complex showed absence of the endothermic peak indicating strong physical interaction between drug and HPβCD in presence of POLO [26].

2.2.2. ATR-FTIR analysis

The probable interaction between QUN and CDs in presence and absence of POLO was studied by ATR-FTIR. The IR spectra recorded for QUN, βCD, HPβCD, POLO and all complexes are shown in Figure 4.

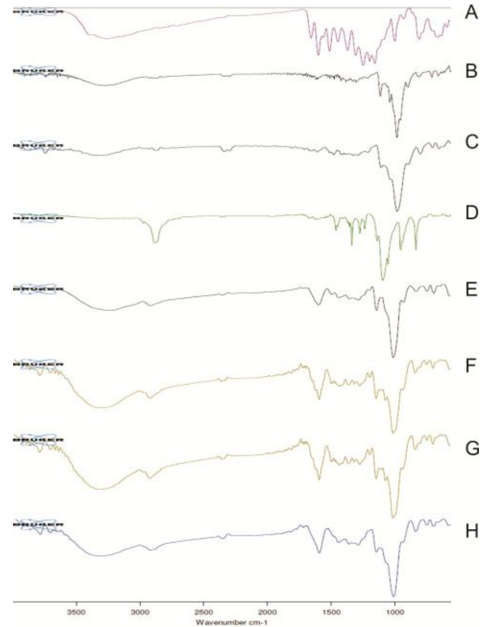

Figure 4. ATR-FTIR spectra of QUN (A), βCD (B), HPβCD (C), POLO (D), QUN-βCD (E) and QUN-HPβCD (F) binary complexes, QUN-βCD-POLO (G) and QUN-HPβCD-POLO (H) ternary complexes.

The principle absorption peaks of QUN were observed at 3390.44 cm^{-1} (O-H stretching vibration of phenol), 3259.7 cm^{-1}, 1660.66 cm^{-1} (C=O aryl ketonic stretch /aromatic ketonic carbonyl stretching), 2841.66 cm^{-1} (C-H stretching), 1603.14 cm^{-1} (C-C aromatic ring stretch), 1374.5 33 cm^{-1} (O-H bending of phenols), 1252.33 cm^{-1} (C-O stretch of aryl ether), 1161.75 cm^{-1} (C-CO-C stretch and bending in ketone) and 812.39 cm^{-1} (C-H bending of aromatic hydrocarbon) [27]. The peaks of βCD were recorded at 3276.49 cm^{-1} (O-H stretch), 1152.61 cm^{-1}, 1078.03 cm^{-1}, 1023.99 cm^{-1} (C- O- C stretch), 997.86 cm^{-1} and 937.10 cm^{-1} whereas absorption peaks of HPβCD were observed at 3737.23 cm^{-1}, 3348.45 cm^{-1} (O-H stretch), 2929.2 cm^{-1} (C- H stretch), 1026.41 cm^{-1} (C-O-C stretch). IR spectrum of POLO shows distinct peaks at 2878.80 cm^{-1} (C-H stretching aliphatic), 1446.23 cm^{-1}, 1341.71 cm^{-1} (in plane O-H bending), 1279.23, 1241.06 and 1100.17 cm^{-1} (C-O stretch).

Alterations in the characteristic bands of QUN and CDs were noted in case of binary and ternary complexes. The peaks in formulations were observed to be either shifted to different wavelengths or smoothened or appeared with decreased intensity. The absence of new peaks indicated non covalent interaction in inclusion complexes. In case of QUN-βCD complex, the peaks of QUN at 3259.70 cm^{-1}, 2841.66 cm^{-1} and 1603.14 cm^{-1}, were shifted to 3240.24 cm^{-1}, 2918.64 $cm^{-1}$and 1599.90 cm^{-1} respectively while other peaks had also shifted and appeared with reduced intensity or disappeared. Broad peak of βCD at 3276.49 cm^{-1} disappeared, while peak at 1023.99 cm^{-1} was shifted to 1018.07 cm^{-1}.

In spectrum of QUN-HPβCD complex, the principle peaks of QUN at 3259.7 cm^{-1} and 2841.66 cm^{-1} shifted to 3299.47 cm^{-1} and 2922.95 cm^{-1} while other peaks were either shifted or appeared with decreased intensity. The prominent peak of HPβCD at 1026.41 cm^{-1} was shifted to 1004.11 cm^{-1}. The disappearance of major peaks of QUN in the spectra of binary complexes clearly indicates the formation of inclusion complex.

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The spectrum of QUN-βCD-POLO ternary complex showed shifting of QUN peaks from 3259.7 cm^{-1} and 2841.66 cm^{-1} to 3254.72 cm^{-1} and 2921.34 cm^{-1} while peak of βCD at 1023.99 cm^{-1} was shifted to 1019.16 cm^{-1}. For QUN-HPβCD-POLO ternary complex, the prominent peaks at 3310.76 cm^{-1} ,2918.55 cm^{-1} ,1596.69 cm^{-1} and 1015.89 cm^{-1} were observed All other peaks had either shifted, disappeared or appeared with less intensity. All these changes confirm the strong physical interaction between QUN and both βCD and HPβCD in the presence of POLO.

2.2.3. XRPD analysis

The XRPD patterns of QUN and all complexes are presented in Figure 5. The diffractogram of QUN exhibited prominent peaks at 12.5, 27.3 and 27.4 (2θ) with peak intensities 784, 969 and 1135 respectively indicating crystalline nature of drug. The peak intensities for βCD at 12.7, 18.9, 20.9 and 27.2 (2θ) recorded were 1751, 2025, 1577, and 1459 respectively. A typical halo-pattern was recorded for HPβCD, which showed peak at 18.3(2θ) with peak intensity 1786 indicating its amorphous nature.

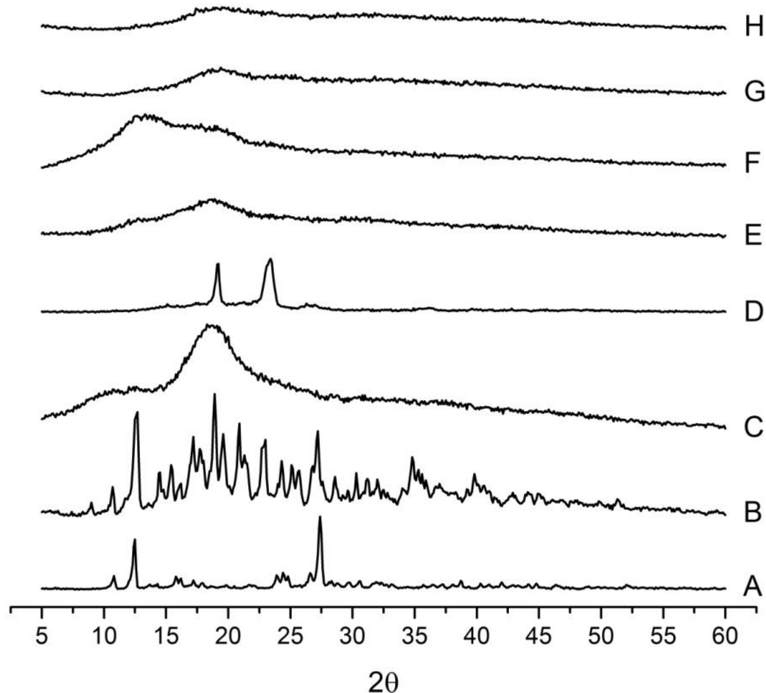

Figure 5. XRPD pattern of QUN (A), βCD (B), HPβCD (C), POLO (D), QUN-βCD (E) and QUN-HPβCD (F) binary complexes, QUN-βCD-POLO (G) and QUN-HPβCD-POLO (H) ternary complexes.

All the prepared inclusion complexes of QUN exhibited characteristic halo pattern and significant reduction in peak intensities, as compared to pure QUN, confirming reduction in the crystallinity of QUN. The intensity of characteristic peak of QUN at 27.4 (2θ) was reduced to 382 and 323 in case of QUN-βCD and QUN-HPβCD respectively. For QUN-βCD-POLO and QUN-HPβCD-POLO ternary complexes, the intensity of the same peak was reduced to 313 and 272 respectively. This indicates that QUN-HPβCD-POLO ternary complex exhibited greater amorphization of QUN. The presence of fine peaks in the spectra of complexes may be due to presence of free uncomplexed QUN to some extent in the complex samples.

2.2.4. SEM analysis

SEM was used to study the surface morphology of pure drug and complexes (Figure 6). The micrographs of pure drug exhibited presence of long cylindrical/needle shaped crystals. The images of freeze dried inclusion complexes showed a significant change in the morphology of the particles. Both change in shape (needle to flaky) and reduction in particle size were observed which indicates an interaction in between QUN and CDs in presence or absence of POLO, resulting into amorphization of QUN. According to the previous reports, a change in the shape of the crystals and aspects of drug indicates its complexation with CD [28,29].

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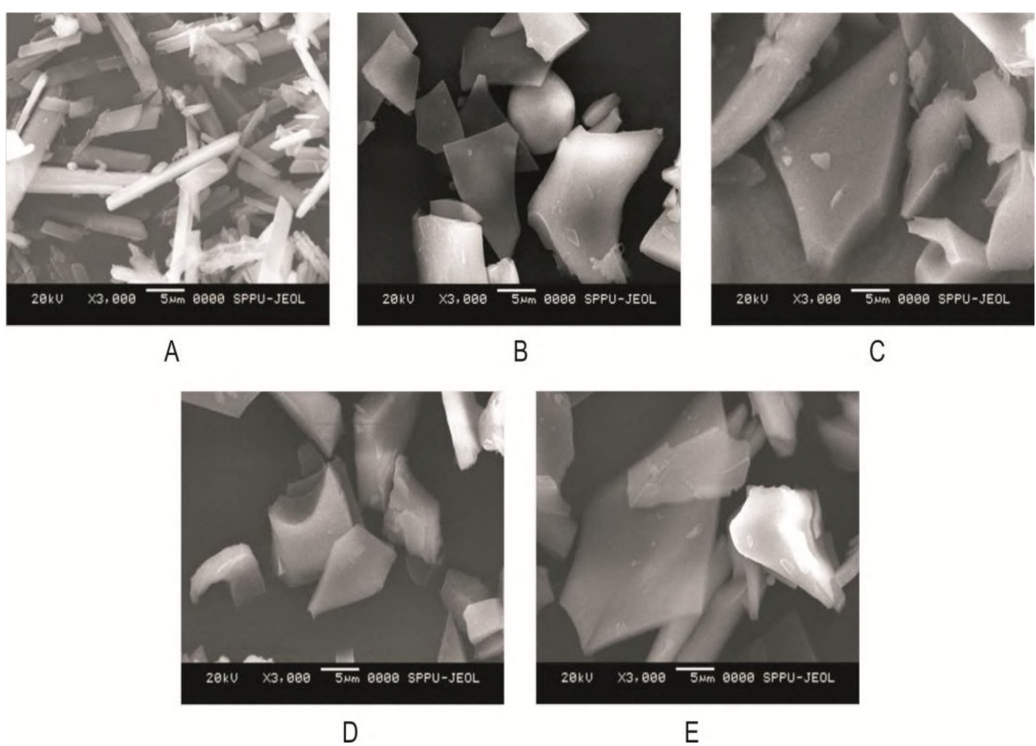

Figure 6. SEM photomicrographs of QUN (A), QUN-βCD (B) and QUN-HPβCD (C) binary complexes, QUN-βCD-POLO (D) and QUN-HPβCD-POLO (E) ternary complexes.

2.3. Percentage drug content and saturation solubility studies

Table 3 presents the drug content (%) and saturation solubility of the physical mixtures and the prepared complexes. The drug content was found to be uniform in all the physical mixtures and complexes ranging within 95.37 to 98.86%.

Table 3. Percentage drug content and saturation solubility of the physical mixtures and complexes

QUN/CD complexes	Drug content (%)*	Solubility in water at 25°C mg/mL*
Pure QUN	-	0.0047± 0.0003
QUN/βCD (PM)	98.73±1.01	0.0071±0.0002
QUN/βCD (FDC)	95.37±1.51	0.0159±0.0002
QUN/βCD/POLO (PM)	97.32±0.85	0.0098±0.0007
QUN/βCD/POLO (FDC)	96.38±0.79	0.0505±0.0142[ab]
QUN/HPβCD (PM)	98.04±1.08	0.0140±0.0060
QUN/HPβCD (FDC)	98.86±1.49	0.0451±0.0019[abc]
QUN/HPβCD/POLO (PM)	97.11±1.22	0.0183±0.0024
QUN/HPβCD/POLO (FDC)	96.81±0.71	0.1602±0.0053[abcd]

* indicates mean of 3 readings + standard deviation
[a] indicates significantly different value as compared to QUN
[b] indicates significantly different value as compared to QUN/βCD (FDC)
[c] indicates significantly different value as compared to QUN/βCD/POLO (FDC)
[d] indicates significantly different value as compared to QUN/HPβCD/POLO (FDC)

The QUN-βCD and QUN-HPβCD binary complexes increased the solubility of QUN by 3.4 and 9.6 folds respectively. On the other hand, QUN-βCD-POLO and QUN-HPβCD-POLO ternary complexes increase the solubility of QUN by 10.7 and 34.1 folds respectively. The physical mixtures showed slight enhancement in the solubility of QUN; however it was significantly less ($p<0.05$) as compared to the complexes containing similar components. The dramatic improvement in the solubility of QUN in case of QUN-HPβCD-POLO ternary complexes can be ascribed to the formation of stable inclusion complex, and reduction in the crystallinity of drug. Besides, micellization of poloxamer chains may also contribute to some extent in enhancing the solubility of QUN.

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2.4. Anti-inflammatory activity

The results for anti-inflammatory activity with respect to change in paw volume and % inhibition of paw inflammation (Mean ± Std. Error) by using Carrageenan-induced rat paw edema method are shown in Figure 7. The positive control i.e. indomethacin decreased the paw volume by 28.07% after two hours. The percent inhibition of paw volume in case of QUN-HPβCD-POLO ternary complex is 44.60% which is more as compared to pure QUN (27.55%), indicating increased anti-inflammatory activity. The increased anti-inflammatory activity confirmed the increase in bioavailability of QUN due to formation of stable inclusion complex between QUN and CDs in presence of POLO and improvement in its solubility.

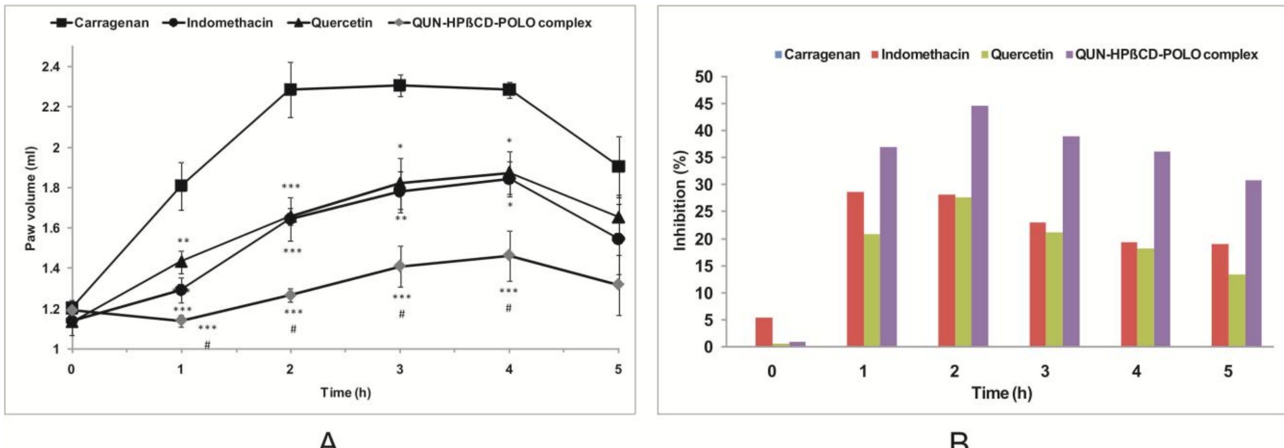

Figure 7. Anti-inflammatory activities of QUN and QUN-HPβCD-POLO ternary complex by Carrageenan-induced rat paw edema method: A- Effect on paw volume (ml); B- Inhibition of inflammation (%). * indicates significant difference of paw volume with respect to control (*- $p<0.05$; **- $p<0.01$; ***- $p<0.001$) and # indicates significant difference of paw volume with respect to QUN treated group (#- $p<0.05$).

3. CONCLUSION

The stability and complexation efficiency of βCD and HPβCD was markedly improved by POLO than the other hydrophilic polymers. QUN-βCD-POLO and QUN-HPβCD-POLO ternary complexes were successfully prepared using the lyophilization method. DSC and ATR-FTIR analysis helped to confirm the formation of inclusion complex whereas XRPD and SEM analysis revealed amorphous nature of the complexes. QUN-HPβCD-POLO complex showed high solubility than the other binary and ternary complexes. An increase in the in vivo anti-inflammatory activity of the QUN-HPβCD-POLO ternary complex clearly indicates enhancement in the bioavailability of QUN.

4. MATERIALS AND METHODS

4.1. Materials

QUN, βCD and polyvinylpyrrolidone K30 (PVP) were purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India. HPβCD (DS: 4.69) was obtained as a gift sample from Gangwal Chemicals, Mumbai, India. Hydroxypropylmethylcellulose E15LV (HPMC) was purchased from Loba Chemie, Mumbai, India and Poloxamer 188 (POLO) was obtained from Signet Chemicals, Mumbai, India. The analytical grade reagents and glass distilled water were used (for the experimental procedures) throughout the experimental procedures.

4.2. Phase solubility studies

The phase solubility studies were performed in distilled water at room temperature (25 ± 2°C) according to the method reported by Higuchi and Connors [16]. The excess quantity of QUN was added to 20 mL of βCD and HPβCD solutions (0 to 0.01 M) in the presence and absence of auxiliary substances (0.5%w/v of HPMC, PVP and POLO). The concentration of polymers was decided on the basis of the preliminary studies. The solubility of QUN in presence of fixed amount of CDs (10mM) and increasing polymer concentration (0.25, 0.5, 0.75, 1, 1.5 and 2%w/v) was determined. The polymer concentration at which maximum QUN was solubilized was selected (i.e. 0.5%w/v). The resultant suspensions were shaken for 72 h at 150 rpm using

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rotary shaker (Lab HOSP, India) to attain equilibrium. Thereafter, suspensions were filtered using Whatman filter paper No. 41, appropriately diluted if necessary and analyzed to determine concentration of QUN using UV-spectrophotometer (Shimadzu 1800, Japan) at 371 nm. The stoichiometry between QUN and CDs was established from the phase solubility curves obtained by plotting the concentration of dissolved QUN (moles/liter) against the respective concentration of CDs (moles/liter). The stability constants (K_S) of the binary and ternary complexes were calculated using the equation [30].

$$K_s = \frac{Slope}{S_0\,(1 - Slope)} \qquad \text{(Eq. 1)}$$

Where, S_0 is the solubility of QUN in distilled water.

The complexation efficiency (C.E.) of CDs was determined by the following equation [30].

$$C.E. = K_s S_0 = \frac{Slope}{(1 - Slope)} \qquad \text{(Eq. 2)}$$

Gibbs free energy change (ΔG_{tr}) in Joules/mole was also calculated to assess the thermodynamics of the solution and complexation process, using the equation

$$\Delta G_{tr} = -2.303\, RT log \frac{S_c}{S_0} \qquad \text{(Eq. 3)}$$

Where, S_c is molar solubility of QUN in aqueous solution of HPβCD or βCD in the presence or absence of hydrophilic polymer, S_0 is molar solubility of QUN in distilled water, R is gas constant and T is temperature in Kelvin.

4.3. Preparation of freeze dried solid inclusion complexes

The method reported by Tayade et al [5] was used for preparation of binary and ternary complexes. The equimolar weighed quantities of QUN and CDs, were dissolved in distilled water (70 mL). To this aqueous solution, 25% ammonia (1.5 mL) was added to dissolve QUN. The resultant solutions were stirred using a magnetic stirrer (2MLH, Remi laboratory Instruments, Mumbai), for 2 h at a room temperature (25 ± 2°C) and were frozen in deep freezer (ELCOLD) at -80°C for 24 hours. The frozen solutions were lyophilized (DELVAC-mimiLyodel) and stored in desiccators till further use.

In case of ternary complexes, same procedure was followed by addition of POLO (0.5 %w/w) in the complexation media. The physical mixtures of binary and ternary systems of QUN, CDs and POLO were prepared in order to compare their solubility with the complexes.

4.4. Characterization of complexes

4.4.1. Differential scanning calorimetry (DSC)

DSC analyzer (TA instuments Q600 SDT USA) was used to perform DSC analysis of QUN, βCD, HPβCD, POLO and all complexes. A sample (5 mg) was sealed in an aluminum pan and subjected to heating at a rate of 10 °C /min from 30–400 °C under nitrogen atmosphere.

4.4.2. Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy(ATR-FTIR)

Attenuated total reflectance (ATR)-Fourier transform infrared spectroscopy (FTIR) (BRUKER–ECO-ATR-ALPHA, Germany) was used for recording infrared spectra of QUN, βCD, HPβCD, POLO and prepared complexes. ATR-FTIR helps in direct analysis of the solid or liquid samples and does not require any complex sample preparation procedure. The samples were directly placed on ATR crystal and analyzed from 600 to 4000 cm^{-1} spectral range with 24 scans.

4.4.3. X-ray powder diffractometry (XRPD)

The XRPD analysis of all samples were performed by using X-ray diffractometer (PW 1723, PHILIPS, Netherland) with tube anode Cu over the interval 05-60° (2θ). Generator tension (voltage) 40 kV, Generator current 30 mA, and scanning speed 2°/min. were maintained during the operation.

4.4.4. Scanning electron microscopy (SEM)

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Scanning electron microscope (SEM-JOEL Instruments, JSM-6360A, Japan) was used for studying surface morphology of QUN and all complexes. Samples were directly mounted on the aluminum stub and coated with a thin gold-ion layer using sputter coated unit an acceleration voltage of 5 kV was applied and the micrographs were examined at ×300, ×1000 and ×3000 magnifications.

4.5. Determination of drug content

The content of QUN in the physical mixtures and complexes was determined by adding the physical mixtures/complexes equivalent to 5 mg of QUN in 50 ml of methanol. The resultant mixtures were stirred on the magnetic stirrer for 3h. The solutions were filtered through Whatman filter paper No. 41, suitably diluted and analyzed spectrophotometrtically at 371 nm.

4.6. Saturation solubility studies

The saturation solubility of the pure QUN, physical mixtures and the complexes was determined using the method described by Higuchi and Connors [16]. Excess quantity of pure QUN and, prepared physical mixtures and complexes were added to the conical flasks each containing 10 ml of distilled water and sealed. The mixtures were shaken using rotary flask shaker for 72 h at room temperature to attain equilibrium. Appropriate fractions were withdrawn and filtered through Whatman filter paper No. 41 and analyzed spectrophotometrically at 371 nm.

4.7. Anti-inflammatory activity

The anti-inflammatory activity of optimized complex of QUN was evaluated in *vivo* as per the approval from Institutional Animal Ethics Committee (IAEC) of College of Pharmacy, Bhor, Maharashtra, India (Approval No. RDCOP/IAEC/Approval/2016-17/03 dated 08/08/2016). Carrageenan-induced rat paw edema method was used as reported previously [31,32].

4.7.1. Experimental design

The Wistar albino rats, 150-180 g of either sex were randomly divided into four groups of six rats in each.

Group	Dose
I . Control	Treated with Normal saline
II. Reference Standard: Indomethacin	10 mg/kg of body weight
III. Pure drug: QUN	10 mg/kg of body weight
IV. Test sample: QUN-HPβCD-POLO	Equivalent to 10 mg of QUN/kg of body weight

To produce acute inflammation, 0.1 ml of 1% freshly prepared carrageenan solution in normal saline was injected in right hind paw of rats by sub-plantar route. Control group rats were treated with normal saline, reference standard group with indomethacin (10mg/Kg body weight per oral), third group with pure QUN 10 mg/ Kg body weight per oral and the test group with QUN-HPβCD-POLO ternary complex; quantity equivalent to 10 mg of QUN/Kg body weight per oral respectively one hour prior to carrageenan injection. The paw volume was measured using plethysmometer at an interval of 1, 2, 3, 4 and 5 hrs after carrageenan injection and increase in mean paw volume in ml (Mean ± Standard Error) and % inhibition of paw inflammation was calculated.

The percentage inhibition of paw inflammation was calculated using the formula:

$$\%\ inhibition\ of\ inflammation = \frac{(Control\ mean - Treated\ mean)}{Control\ mean} \times 100 \quad \text{(Eq. 4)}$$

4.8. Statistical analysis

All values were shown as Mean ± Standard Error Mean (S.E.M.). Statistical analysis was performed using one-way analysis of variance (ANOVA). $P<0.05$ was considered statistically significant.

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Author contributions: Concept – A.K., R.D.; Design – A.K., R.D.,; Supervision – R.D.; Materials – A.K; Data Collection and/or Processing – A.K., R.D.; Analysis and/or Interpretation – A.K., R.D., V.G.; Literature Search – A.K., R.D., V.G.; Writing – A.K.; Critical Reviews – R.D., V.G., A.K.

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ESTIMATION OF HEAVY METALS FROM SHANKH-VATI TABLET

Suvarna S. Dhebe[1]*, Avinash M. Bhagwat[1], Satish S. Deshpande[2] and Sunil V. Garad[3]

[1]Yashoda Shikshan Prasarak Mandal's, Yashoda Technical Campus, Faculty of Pharmacy (B. Pharm), Wadhe, Satara- 415011, Maharashtra, India.

[2]Production Manager, Ayurvedeeya Arkshala Ltd; Satara – 415 002, Maharashtra, India.

[3]Associate Professor, Maharashtra College of Pharmacy, Nilanga, Dist- Latur 413521.

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***Corresponding Author**
Suvarna S. Dhebe
Yashoda Shikshan Prasarak Mandal's, Yashoda Technical Campus, Faculty of Pharmacy (B. Pharm), Wadhe, Satara- 415011, Maharashtra, India.

ABSTRACT

Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed. Eight common heavy metals are discussed in this brief: arsenic, barium, cadmium, chromium, lead, mercury, selenium, and silver. Even these metals do not have any biological role, they remain present in our body harms human body and its functioning. As all ayurvedic formulation contains large amount of heavy metals due to the herbal ingredients used for preparation. One of the ayurvedic formulation from among formulation i.e. Shankh-vati tablet. We have studied the amount of heavy metals present in shankh-vati tablet. Shankh-vati is and ayurvedic classical formulation available in tablet form which is used to manage the digestive disorders. It is also used to resolve the various problems like anorexia, vomiting, gastritis etc. Natural ingredients used in this medicine help to balance the tridoshas in body. Shankh-vati totally contains 15ingredients. Mainly parad (mercury) is mostly used in ayurvedic formulation. Mercury is present in large amount in ayurvedic formulation. Along with determination of heavy metals, limit test for iron and sulfate is also done. Sulfate occur as microscopic particles(aerosols) resulting from fossil fuel and biomass combustion. They increase the acidity of the atmosphere and form acid rain. The limit test has passed which confirms that heavy metals in shankh-vati tablet is in limit. Also other metals like iron and sulfate are in limit.

KEYWORDS: Shankh-vati Tablet, Heavy Metals, Toxicity, Mechanisms of Heavy Metals, Limit tests.

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INTRODUCTION

Heavy Metals

A metal of relatively high density (specific gravity greater than about 5 g/cm^3) or of high relative atomic weight is defined as a heavy metal. The term "Heavy metals" is used to describe more than a dozen elements that are metals or metalloids ex; chromium, arsenic, cadmium, lead, mercury, manganese, etc. Heavy metals are natural constituents of the Earth's crust. Heavy metals cannot be destroyed nor degraded. In small amounts, they enter the human body via food, drinking water and air. Living organisms require varying amounts of "heavy metals". Iron, cobalt, copper, manganese, molybdenum, and zinc are required by humans. Therefore, heavy metals can be described as any metallic element that has a relatively high density and is toxic or poisonous at low concentrations. Human activities affect the natural geological and biological distribution of heavy metals through pollution of air, water, and soil. Heavy metals which are toxic are realsed by humans only. Bioaccumulation refers to an increase in the concentration o f a metal in a biological organism over time, compared to the normal concentration in the environment.[25] Some heavy metals like mercury and lead are toxic metals that have no known vital or beneficial effect on organisms, and their accumulation over time in the bodies of animals can cause serious illness of harmful effects. Certain elements that are normally toxic are, for certain organisms or under certain conditions, may be beneficial. Therefore, they tend to accumulate in the soil, seawater, freshwater, and sediments. In small quantities, certain heavy metals are nutritionally essential for a healthy life (e.g., iron, copper, manganese, and zinc). Some of these are referred to as the trace elements. These elements, or some form of them, are commonly found naturally in foodstuffs, in fruitsand vegetables, and in commercially available multivitamin products.As some ayurvedic formulation also includes needed essential heavy metals. The ayurvedic formulations contain required amount of the essential heavy metals which are useful for lives. There are many types of different ayurvedic formulations in which all of them contains heavy metals. Although it is acknowledged that heavy metals have many adverse health effects and last for a long period of time, heavy metal exposure continues is increasing in many parts of the world. Heavy metals are significant environmental pollutants and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons. The most commonly found heavy metals in waste water include arsenic, cadmium, chromium, copper, lead, nickel, and zinc, all of which cause risks for human health and the environment. Various sources of heavy metals include soil erosion, natural weathering of the earth's crust, mining, industrial

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effluents, urban runoff, sewage discharge, insect or disease control agents applied to crops, and many others.[24] These metals bind with protein sites which are not made for them by displacing original metals from their natural binding sites causing malfunctioning of cells and ultimately toxicity. Previous research has found that oxidative deterioration of biological macromolecules is primarily due to binding of heavy metals to the DNA and the nuclear proteins.[11]

Shankh-vati is an Ayurvedic formulation or medicine available in tablet form which is used to manage the digestive disorders. Calmative action of shankh-vati helps to provide relief in the burning sensation of stomach, also helps to improve the body's ability to absorbs the nutrition in a natural way. It is mainly prepared by triturating the fine powder of ingredients with lemon juice to prepare a paste. From which paste, tablets are prepared, dried and stored.

The toxic effects of these metals, even though they do not have any biological role, remain present in some or the other form harmful for the human body and its proper functioning. They sometimes act as a pseudo element of the body while at certain times they may even interfere with metabolic processes.

Various public health measures have been undertaken to control, prevent and treat metal toxicity occurring at various levels, such as occupational exposure, accidents and environmental factors.

Physiological roles of heavy metals in humans

- Iron – hemoglobin, myoglobin
- Cobalt – coenzyme
- Copper – co-factor in enzymes
- Zinc – in enzymes
- Selenium – in enzymes
- Chromium – Cr^{3+} in enzyme

Uses of heavy metals

- Mercury is found in batteries, dental amalgam, vacuum pumps and valves. Airborne mercurycomes from the combustion of diesel, jet fuel and heating oil.

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- Arsenic is high in seafood and may also be found as a contaminant in animal feeds. It is also present in wood preservatives, herbicides, corrosion inhibitors, in lead and copper alloys.
- Cadmium is used industrially as an anti-friction agent, as a rust-proofer, in plastics manufacture, in alloys and in alkaline storage batteries.
- Chromium is found in fresh foods, copy machine toner and nickel in coins, kitchen utensils and milk.
- Copper is essential to all living organisms and has a wide range of effects depending on concentration and chemical formulation. It is used in the electrical industry in alloys such as brass, in chemical catalysts and in wood-preservatives.
- Lead has been used in batteries, electronic equipment's, in petrol, toys, paint, etc. Lead has been used as fuel additive in many countries for several years, although this practice has since stopped in most of the countries of the world, because of the health implications of lead.
- Manganese compounds are used in manufacturing of products such as batteries, steel and unleaded petrol. Manganese dioxide is commonly used in the production of dry-cellbatteries, matches, fireworks, porcelain and glass-bonding materials. It is also used as thestarting material for the production of other manganese compounds.
- Manganese chloride is a precursor of other manganese compounds. It is used as a catalyst inthe chlorination of organic compounds, in animal feed to supply essential trace minerals andin dry-cell batteries. Manganese sulfate is used as a fertilizer, livestock nutritional supplement and in ceramics.

Effects of heavy metals on human

Humans are always exposed to the natural levels of trace elements. Under normal circumstances; the body is able to control some of these amounts. However, continuous exposure to elevated levels of metals could cause serious illness or death. Increased exposure is due to contaminated soil and other industrial wastes which affects the skin.

There are 35 metals that are of concern for us because of residential or occupational exposure, out of which 23 are heavy metals: antimony, arsenic, bismuth, cadmium, cerium, chromium, cobalt, copper, gallium, gold, iron, lead, manganese, mercury, nickel, platinum, silver, tellurium, thallium, tin, uranium, vanadium, and zinc. These heavy metals are

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commonly found in the environment and diet. In small amount they are required for maintaining good health but in larger amounts they can become toxic or dangerous.

The health hazards presented by heavy metals depend on the level and the length of exposure. In some cases, the health effects are immediately apparent; in others, the effects are delayed.

High levels of toxic metals deposited in body tissues and subsequently in the brain, may cause significant developmental and neurological damage, including depression,increased irritability, anxiety, insomnia, hallucination, memory loss, aggression and many other disorders. Heavy metal toxicity can lower energy levels and damage the functioning of the brain, lungs, kidney, liver, blood composition and other important organs.

MATERIALS AND METHODS

Shankh-Vati

Shankh-vati, an ayurvedic medicine available in tablet form which is used to manage the digestive disorders. This remedy is quite effective to improve the digestive fire in the body and also helps to strengthen the digestive system. It is quite good to resolve the various problems like anorexia, vomiting, gastritis etc. For anorexia, it helps to improve the digestion and appetite as well. Natural ingredients, used in this medicine help to balance the tridoshas in body. Works well in the problem of excessive gas, flatulence, burping etc. Calmative action of Shankh-vati helps to provide relief in the burning sensation of stomach, also helps to improve the body's ability to absorb the nutrition in a natural way.

It contains heavy metals ingredients and hence should only be used under strict medical supervision. 15 varieties of shankh-vati were found available across different across different Ayurveda texts. Shankha bhasma, Hingu, Vatsanabha, Trikatu, Kshara and Lavana are the ingredients common to most varieties of Shankha Vati.Shankh-vati is a classical Ayurvedic formulation that is widely used by the practitioners of Ayurveda, contains a heavy metal i.e. Parad(mercury) and a poisonous herbal drug i.e. Vatsanabha (Aconitum chasmantum Staffex Holmes) along with other ingredients. There are certain apprehensions on this formulation, mainly due to the presence of its ingredients Vatsanabha Shankha Vati(Bhaishajya Ratnavali 10/186-187, also in Ayurvedic Formulary of India part 1), is one of the formulations in which Vatsanabha is used without its usual coingredient and antidote Tankana (Borax).

Fine powder of each of

Panchalavana\Ingredients

1. Saindhava Lavana- Rock salt
2. Sauvarchala Lavana- Sochal salt
3. Samudra Lavana- Common salt
4. Vida Lavana- Vida salt
5. Nimbu swarasa- Lemon juice- Quantity Sufficient
6. Shankha Bhasma- Bhasma of Conch Shell
7. Hingu- Asafoetida
8. Shunti- Ginger Rhizome – *Zingiber officinalis*
9. Maricha- Black pepper- *Piper nigrum*
10. Pippali- Long pepper fruit- *Piper longum*
11. Purified Mercury- Parad
12. Vatsanabha- Aconitum ferox
13. Shuddha Gandhaka
14. Krishna Lavana

Uses

1. Dyspepsia
2. Low digestion power
3. Anorexia
4. Peptic diseases
5. Emaciation
6. Improper digestion
7. Excessive gas
8. Gulma
9. Burning sensation in stomach

Dosage

1-2 tablets (250-500 mg) once or twice in a day, before or after food or as directed by ayurvedic doctor.

Precautions[9]

1. This medicine should be strictly taken under the medical supervision.
2. It should be avoided by pregnant, lactating mothers and children.

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3. Over dosage may cause slight burning sensation in stomach.
4. For high BP patients this medicine should be taken with care as it contains salt as an ingredient.
5. Keep out of reach and sight of children.
6. Store in a cool dry place.

Mercury

The metallic mercury is a naturally occurring metal which is a shiny silver-white, odorless liquid and becomes colorless and odorless gas when heated. Mercury is very toxic and exceedingly bio accumulative. Major sources of mercury pollution include anthropogenic activities such as agriculture, municipal wastewater discharges, mining, incineration, and discharges of industrial wastewater.[10]

Mercury exists mainly in three forms

1. Metallic elements
2. Inorganic salt
3. Organic compounds

Each of which possesses different toxicity and bioavailability. These forms of mercury are present widely in water resources such as lakes, rivers and oceans where they are taken up by microorganisms and get transformed into methyl mercury within the microorganism, eventually undergoing bio magnifications causing significant disturbance to aquatic lives. Consumption of this contaminated aquatic animal is the major route of human exposure to methyl mercury. Mercury is extensively used in thermometers, barometers etc. Mercury poisoning symptoms include blindness, deafness, brain damage, digestive problems, kidney damage, lack of coordination and mental retardation. One of the most famous cases of mercury poisoning resulting from chronic exposure was the disaster that occurred in Minamata, Japan, where methylmercury was discharged from a plastics manufacturing plant into the waters of Minamata Bay in the 50s and 60s. This is reflected in its abundance in the earth's crust as oxides, carbonates, silicates with iron, magnesium and as sulphides, arsenide's and telurides. Nickel salts are soluble and can occur as a leachate from nickel bearing rocks.[20] It is also a known 'carcinogen'.

Mercury is considered the most toxic heavy metal in the environment. Mercury poisoning is referred to as acrodynia or pink disease. Mercury is released into the environment by the

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activities of various industries such as pharmaceutical, papers and pulp preservatives, agriculture industry, and chlorine and caustic soda production industry. Mercury has ability to combine with other elements and form organic and inorganic mercury. Exposure to elevated levels of metallic, organic, and inorganic mercury can damage brain, kidneys and the developing fetus. Mercury is present in most foods and beverages in the range <1 to 50ug/kg. In marine foods it is often seen at higher levels.

Micro-organisms convert the mercury present in soil and water into methyl mercury, a toxin which can accumulate with fish age and with increasing trophic levels. EPA has declared mercuric chloride and methyl mercury to be highly carcinogenic. The nervous system is very sensitive to all types of mercury. Increased exposure of mercury can alter brain functions and lead to shyness, tremors, memory problems, irritability, and changes in vision or hearing.

Exposure to metallic mercury vapors at higher levels for shorter periods of time can lead to

Damage to lungs, Vomiting, Diarrhea, Nausea, Skin rashes, Increased heart rate or blood pressure.

Symptoms of organic mercury poisoning include

1. Depression
2. Memory problems
3. Tremors
4. Fatigue
5. Headache
6. Hair loss etc.

Since these symptoms are common also in other conditions, it may be difficult to diagnose such cases. Due to the excess health effects associated with exposure to mercury, the present standard for drinking water has been set at lower levels of 0.002 mg/L and 0.001mg/L by the Environmental Protection Act and World Health Organization.

Mechanism of mercury toxicity

The brain remains the target organ for mercury, yet it can impair any organ and lead to malfunctioning of nerves, kidneys and muscles. It can cause disruption to the membrane potential and interrupt with intracellular calcium homeostasis. Mercury binds to freely

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available thiols as the stability constants are high. Mercury vapours can cause bronchitis, asthma and temporary respiratory problems. Mercury plays a key role in damaging the tertiary and quaternary protein structure and alters the cellular function by attaching to the selenohydryl and sulfhydryl groups which undergo reaction with methyl mercury and hammer the cellular structure. It also intervenes with the process of transcription and translation resulting in the disappearance of ribosomes and eradication of endoplasmic reticulum and the activity of natural killer cells. The cellular integrity is also affected causing free radical formation. The basis for heavy metal chelation is that even though the mercury sulfhydryl bond is stable and divided to surrounding sulfhydryl consisting ligands, it also contributes free sulfhydryl groups to promote metal mobility within the ligands.[4]

Procedure[2]

1. Take powder 0.5gmof tablet, treat it with 7 ml of concentrated nitric acid, add 15ml concentrated sulphuric acid in kjeldahl flask.
2. Heat the mixture under reflux gently strongly for 30min.
3. Cool the mixture and add 50ml of concentrated nitric acid to remove brown fumes.
4. Continue the addition of concentrated nitric acid and boiling until liquid is colourless. Cool, wash the condenser with 100ml of water.
5. Remove flask add 1.0% potassium permanganate solution dropwise until pink colour persist.
6. Decolorize it by adding 6.0% hydrogen peroxide dropwise to remove excess potassium permanganate followed by 3.0ml concentrated nitric acid.
7. Titrate with 0.1N ammonium thiocyanate solution using ferric alum as indicator.

Each ml of 0.1N ammonium thiocyanate solution is equivalent to 0.01003gm mercury.

Observation table

0.1N Ammonium thiocyanate as titrant	
Burette reading	**Mean(ml)**
2.5ml	2.5ml
2.5ml	
2.5ml	

Factor: - As each ml of 0.1N ammonium thiocyanate solution is equivalent to 0.01003gm of mercury.

2.5ml of 0.1N ammonium thiocyanate contains 0.025015gm of mercury.

Iron

Iron is the second most abundant metal on the earth's crust. Iron is the most abundant transition metal in the earth's crust. Biologically it is the most important nutrient for most living creatures as it is the cofactor for many vital proteins and enzymes. Iron occupies the 26th elemental position in the periodic table. Iron is a most crucial element for growth and survival of almost all living organism. It is one of the vital components of organisms like algae and of enzymes such as cytochromes and catalase, as well as of oxygen transporting proteins, such as hemoglobin and myoglobin. Iron is an attractive transition metal for various biological redox processes due to its inter-conversion between ferrous and ferric ions. The source of iron in surface water is anthropogenic and is related to mining activities.

Iron occurs as particulate ferric hydroxide or in the form of organometallic compound in natural system. Fe (III) oxide and Fe (II) are ubiquitous in anoxic environments and they affect the distribution, transport, and biogeochemistry of chemical contaminants by absorption onto Fe(III) oxides and by control of oxidation and reduction reactions. Iron is regarded as one of the essential elements for humans. Approximately 3000 to5000 mg of iron exists in the human body. Therefore, as long as the quantity of iron in the environment is not too large, it may not be harmful to the human body.[1]

However, iron can cause undesirable problems in industrial processes or ecosystems if itsconcentration in water is not managed properly. WHO has recommended a value of 0.3mg/L as permissible limit for drinking water. For fresh Chronic excessive intake of iron may lead to hemosioderosis or hemochromatosis. Iron constitutes about 4.7 % of the earth's crust. It is the second most importantmetallic element in the terrestrial environment. Iron is extremely useful, but can also behighly toxic to cellular constituents when present in excess. Iron is an important part of theplant's oxidation- reduction reactions. Iron is a structural component of cytochromes, hemes, and numerous otherelectron-transfer systems, including nitrogenase enzymes necessary for the fixation ofdinitrogen gas. The major problem with iron availability is how to keep iron sufficientlysoluble for plants to absorb enough of it. In strongly acidic solutions (pH < 5), iron becomesincreasingly soluble, and is rarely deficient It is essential for the physiological processes ofall living organisms.

The production of sulphuric acid and the discharge of ferrous takes place due oxidation of iron pyrites that are common in coal seams. The concentration of dissolved iron in the deep ocean is normally 0.6nM or 33.5x10-9 mg/L. In freshwater the concentration is very low with

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a detection level of 5 ug/L-ICP, whereas in groundwater the concentration of dissolved iron is very high with 20 mg/L.A study of iron toxicity on aquatic plants, particularly rice, reported that the growth of species of aquatic reed was found to be inhibited by concentration of 1 mg/L total iron. Acid soils restrict rice production and together with Zn deficiency cause a macronutrient disorder in wetland rice. The production of lowland rice was greatly affected by high concentrations of reduced iron in the flooded soils. The features of iron toxicity in rice include high uptake of fe2+ by roots, acropetal translocation into leaves, bronzing of rice leaves and yield loss.[20]

Children's are highly susceptible to iron toxicity as they are exposed to a maximum of iron-containing products. Iron toxicosis occurs in four stages;

- First stage: This stage occurs after 6 hours of iron overdose is marked by gastrointestinal effects such as gastrointestinal bleeding, vomiting and diarrhea.
- Second stage: The second stage progresses within 2 to 4hrs of overdose and it is considered as the latent period, a period of apparent medical recovery.
- Third stage: The third stage occurs between 2 to 24 hrs. After the onset of certain clinical symptoms. This stage is characterized by shocks, hypotension, lethargy, tachycardia, hepatic necrosis, metabolic acidosis and sometimes death.
- Fourth stage: The fourth stage occurs within 12 to 24hrs of iron overdose. This stage is marked by the formation of gastrointestinal ulcerations and development of strictures.
- Fifth stage: These stage occurs within 1 to 7weeks.

Mechanism of iron toxicity

A wide range of harmful free radicals are formed when the absorbed iron fails to bind to the protein, which in turn severely affects the concentration of iron in mammalian cells and biological fluids. This circulating unbound iron results in corrosive effect of the gastrointestinal tract and biological fluids. An extremely higher level of iron enters into the body crossing the rate-limiting absorption step becomes saturated. These free irons penetrate into cells of the heart, liver and brain. Due to the disruption of oxidative phosphorylation by free iron, the ferrous iron is converted to ferric iron that releases hydrogen ions, thus increasing metabolic acidity. The free iron can also lead to lipid peroxidation, which results in severe damage to mitochondria, microsomes and other cellular organelles. The toxicity of iron on cells has led to iron mediated tissue damage involving cellular oxidizing and reducing mechanisms and their toxicity towards intracellular organelles such as mitochondria and

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lysosomes. A wide range of free radicals that are believed to cause potential cellular damage are produced by excess intake of iron. The iron produced hydrogen free radicals attack DNA, resulting in cellular damage, mutation and malignant transformations which in turn cause an array of diseases.[1]

Procedure[31]

Standard

1. Take 2.0ml of iron standard solution (20 ppm fe) in labeled Nessler's cylinder (S).
2. Add 2ml of a 20% w/v solution of iron free citric acid.
3. And add 0.1ml of thioglycollic acid, mix well, make alkaline with iron free ammonia solution.
4. Dilute to 50ml with water and allow to stand for 5 minutes.

Test

1. Weigh accurately 2.5g of zinc sulphate and dissolve in sufficient carbon dioxide free water to produce 50ml in a beaker. Take 2.0ml of solution and diluted to 10ml with water in a labeled Nessler's cylinder(T).
2. Add 2ml of a 20% w/v solution of iron-free citric acid.
3. And add 0.1ml of thioglycollic acid, mix well and make alkaline with iron free ammonia solution.
4. Dilute to 50ml with water and allow to stand for 5minute.
5. View the colour intensity against white background and compare with that of standard.

Observation: The colour intensity produced in test solution is **same** as the colour intensity produced in standard solution.

Inference: -The given sample of shankh-vati tablet complies the limit for iron.

Lead

Lead is a highly toxic metal whose widespread use has caused extensive environmental contamination and health problems in many parts of the world. Lead is a bright silvery metal, slightly bluish in a dry atmosphere. It begins to tarnish on contact with air, thereby forming a complex mixture of compounds, depending on the given conditions. The sources of lead exposure include mainly industrial processes, food and smoking, drinking water and domestic sources. The sources of lead were gasoline and house paint, which has been extended to lead bullets, plumbing pipes, pewter pitchers, storage batteries, toys and faucets. In the US, more

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than 100 to 200,000 tons of lead per year is being released from vehicle exhausts. Some is taken up by plants, fixation to soil and flow into water bodies, hence human exposure of lead in the general population is either due to food or drinking water.

Lead is extremely toxic heavy metal that disturbs various plant physiological processes and unlike other metals, such as zinc, copper and manganese, it does not play any biological functions. A plant with high lead concentration fastens the production of reactive oxygen species(ROS), causing lipid membrane damage of chlorophyll and photosynthetic processes and suppresses the overall growth of the plant. Metallic lead does not dissolve in water and does not bum, however, lead can combine with other chemicals to form lead compounds or lead salts. Some lead salts dissolve in water better than others. Although lead itself cannot be broken down, lead compounds in water may combine with different chemicals depending on the acidity and temperature of the water.

Most of the lead used by industry comes from mined ores (primary) or from recycled scrap metal or batteries (secondary). The main sources of lead pollution are lead smelters, battery manufacturers, paper and pulp industries, boat and ship fuels and ammunition industries. In addition, the production of television picture tubes, pigments, petroleum fuels, printing, glass industries, photographic materials, etc., also adds Pb(II) to the environment. People living near hazardous waste sites may be exposed to lead by breathing air, drinking water, eating foods, or swallowing or touching dust or dirt that contains lead.

Cigarette smoke also contains small amounts of lead. Lead may enter foods if they are put into improperly glazed pottery or ceramic dishes and from leaded-crystal glassware.

Hypertension has also been associated with lead exposure in the general population. At the typical levels to which individuals are exposed, lead can cross the placenta and damage developing fatal nervous systems. High level exposure to lead may cause miscarriage in pregnant woman and can also damage the organs responsible for sperm production in male.

The most severe neurological effect of lead in adults is lead encephalopathy, which is a general term to describe various diseases that affect brain function. Lead exposure may cause weakness in fingers, wrists, or ankles.

Children are more sensitive to the effects of lead than adults. A child who swallows large amounts of lead may develop blood anemia, kidney damage, severe stomachache, muscle

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weakness, and brain damage. The lower IQ levels and other neuropsychological deficiencies among the children exposed to higher lead levels have been well documented. Lead acetate and lead phosphate have been shown to be potential carcinogens based on studies in animals.

Bioavailability is defined as the fraction of the element from an ingested matrix such as soil, water or food that can be absorbed by an organism. The bioavailability and environmental mobility of the metals are dependent upon the form in which the metal is associated with the soil.

Lead is used for production of batteries, cosmetics, metal products such as ammunitions, solder and pipes. Lead is highly toxic and hence its use in various products, such as paints, gasoline has been considerably reduced nowadays.[8]

The main sources of lead exposure are- Lead based paints, Gasoline, Cosmetics, Toys, Household, dust, Contaminated, soil, Industrial emissions etc.

Lead poisoning was considered to be a classic disease and the signs that were seen in children and adults were mainly pertaining to the central nervous system and the gastrointestinal tract. Lead poisoning can also occur from drinking water. The pipes that carry the water may be made of lead and its compounds which can contaminate the water. According to the Environmental Protection Agency (EPA), lead is considered a carcinogen. Lead has major effects on different parts of the body. Lead distribution in the body initially depends on the blood flow into various tissues and almost 95% of lead is deposited in the form of insoluble phosphate in skeletal bones.

Toxicity of lead, also called poisoning, can be either acute or chronic. Acute exposure can cause: Loss of appetite, Headache, Hypertension, Abdominal pain, Renal Dysfunction, Fatigue, Sleeplessness, Arthritis, Hallucinations and Vertigo etc.

Chronic exposure of lead can result into; Mental Retardation, Birth defects, Psychosis, Autism, Allergies, Dyslexia, Weight loss, Hyperactivity, Paralysis, Muscular Weakness, Brain Damage, Kidney damage and may even cause death.

Mechanism of lead toxicity

Lead metal causes toxicity in living cells by following ionic mechanism and that of oxidative stress. Oxidative stress in living cells is caused by the imbalance between the production of

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free radicals and the generation of antioxidants to detoxify the reactive intermediates or to repair the resulting damage. Antioxidants, as e.g.; glutathione, present in the cell protect it from free radicals such as hydrogen peroxide. Under the influence of lead, however, the level of the ROS increase and the level of antioxidants decreases. Since glutathione exists both in reduced (GSH) and oxidized(GSSG) state, the reduced form of glutathione gives its reducing equivalents from its thiol groups of cysteine to ROS in order to make them stable. In the presence of the enzyme glutathione peroxidase, reduced glutathione readily binds with another molecule of glutathione after donating the electron and forms glutathione disulfide(GSSG). The reduced from (GSH) of glutathione accounts for 90% of the total glutathione content and the oxidized from(GSSG) accounts for 10% under normal conditions. Yet under the condition of oxidative stress, the concentration of GSSG exceeds the concentration of GSH. Another biomarker for oxidative stress is lipid peroxidation, since the free radical collects electron from lipid molecules present inside the cell membrane, which eventually causes lipid peroxidation. At very high concentrations, ROS may cause structural damage to cells, proteins, nucleic acid, membranes and lipids, resulting in a stressed situation at cellular level.

The ionic mechanism of lead toxicity occurs mainly due to the ability of lead metal ions to replace other bivalent cations like Ca, Mg, Fe and monovalent cations like Na, which ultimately disturbs the biological metabolism of the cell. The ionic mechanism of lead toxicity causes significant changes in various biological processes such as cell adhesion, intra and inter cellular signaling, protein folding, maturation, apoptosis, ionic transportation, enzyme regulation, and release of neurotransmitters. Lead can substitute calcium even in picomolar concentration affecting protein kinase C, which regulates neural excitation and memory storage.[14]

Procedure[31]

Standard

1. In a 50ml labeled Nessler's cylinder(S) Pippete 1.0ml of lead standard solution (20 ppm Pb).
2. Dilute with distilled water to 25ml.
3. Adjust the pH with dilute acetic acid or dilute ammonia solution in between 3.0 to 4.0.
4. Dilute with distilled water about 35ml and mix with glass rod.
5. Add 10ml of freshly prepared hydrogen sulphide solution.

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6. Mix and dilute to 50ml with water.
7. Allow to stand for 5 minutes.

Test

1. Accurately weigh 4g of sodium chloride and add to labeled Nessler's cylinder(t).
2. Add 2ml of dilute acetic acid and mix well, then add sufficient water to produce 25ml.
3. Adjust the p^H with dilute acetic acid or dilute ammonia solution in between3.0 to 4.0.
4. Dilute with water to 35ml and mix well.
5. Add 10ml of freshly prepared hydrogen sulphide solution.
6. Mix and dilute to 50ml with water.
7. Allow to stand for 5 minutes.
8. View downwards over a white surface and compare with that of standard.

Observation: -The colour intensity produced in test solution is **less** than the colour intensity produced in standard solution.

Inference: -The given sample of Shankh-vati tablet complies the limit for heavy metals as per the Indian Pharmacopeia.

Sulfate

- White vitriol is zinc, sulfate heptahydrate. Some sulphates were known to alchemists. The vitriol salts, from the Latin vitreolum, glassy, were so-called because they were some of the first transparent crystals known.
- Green vitriol is iron, sulfate heptahydrate.
- Blue vitriol is copper, sulfate pentahydrate.
- Alum, a double sulfate of potassium and aluminium.

The sulfate anion consists of a central sulfur atom surrounded by four equivalent oxygen atoms in a tetrahedral arrangement. The symmetry is the same as that of methane. The sulfur atom is in the +6 oxidation state while the four oxygen atoms are each in the -2 state. The sulfate ion carries an overall charge of -2 and it is the conjugate base of the bisulfate ion, which is in turn the conjugate base of sulfuric acid. Organic sulfate esters, such as dimethylsulfate, are covalent compounds and esters of sulfuric acid. The tetrahedral molecular geometry of the sulfate ion is as predicted by VSEPR theory.

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Sulfates are widely used by industries. Major compounds include;

- Gypsum, the natural mineral form of hydrated calcium sulfate, is used to produce plaster. About 100 million tons per year are used by the construction industry.
- Copper sulfate, a common algaecide, the more stable form is used for galvanic cells as electrolyte.
- Iron sulfate, a common form of iron in mineral supplements for humans, animals, and soil for plants.
- Magnesium sulfate(commonly known as Epsom salts), used in therapeutic baths.
- Lead sulfate, produced on both plates during the discharge of a lead-acid battery.
- Sodium Laureth Sulfate, or SLES, a common detergent in shampoo formulation.[26]

Procedure[31]

Standard

1. Take 1ml of 25% w/v solution of barium chloride in the Nessler's cylinder(S).
2. Add 1.5ml of ethanolic sulphate standard solution (10 ppm SO_4)mix and allow to stand for 1minute.
3. Add 0.15ml of 5M acetic acid.
4. Add sufficient water to produce 50ml, stir immediately with glass rod.
5. Allow to stand for 5 minutes.

Test

1. Take 1ml of 25% w/v solution of barium chloride in the labeled Nessler's cylinder (T).
2. Add 1.5ml of ethanolic sulphate standard solution (10 ppm SO_4) mix and allow to stand for 1minute.
3. Weigh accurately 1.0g of sodium bicarbonate and add to a labeled Nessler's cylinder.
4. Add 10ml distilled water, neutralize with hydrochloric acid and dilute to 15ml with distilled water.
5. Add 0.15ml of 5M acetic acid.
6. Add sufficient water to produce 50ml, stir immediately with glass rod and allow to stand for 5 minutes.
7. View transversely against a black background.
8. Compare opalescence with that of standard solution.

Observation: -The colour intensity produced in test solution is **less** as the colour intensity produced in standard solution.

Inference: -The given sample of Shankh-vati tablet complies the limit for sulphate as per the Indian Pharmacopeia.

RESULTS

1. 0.5gm of drug contains 0.025015gm of mercury, which is within limit.
2. The given sample of shankh-vati tablet complies the limit for iron.
3. The given sample of shankh-vati tablet complies the limit for heavy metals as per Indian pharmacopeia.
4. The given sample of shankh-vati tablet complies the limit for sulfate as per Indian pharmacopeia.

DISCUSSION

Shankh-vati Tablets were collected as a gift sample from Ayurvedeeya Arkashala Ltd. Satara, to determine whether the heavy metals like mercury i.e. also known as “Parad” in Ayurveda and lead is in limit or not. As we know many patients take ayurvedic medications which contains heavy metals, due to herbal plants used for preparations. We know, heavy metals have toxic and dangerous effects on human health. They produce threat to life and effects the body functions.

Determination of mercury is done by the procedure mentioned in ayurvedic pharmacopeia. Other metals are detected by limit test for heavy metals. As by results we are able to know that heavy metals are within limit in shankh-vati tablet. As all limit tests complies with standard solution.

CONCLUSION

By overall study, we concluded that Shankh-vati tablet an ayurvedic formulation contains heavy metals(mercury and lead) and other metals like (Iron and Sulfate) within limit. So, it cannot produce any toxic effects on consumer.

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AN EMPERICAL STUDY ON THE EMPLOYABILITY SKILLS OF PHARMACY UNDERGRADUATES IN SATARA REGION

Pratik R. Bhosale*, Avinash M. Bhagwat and Sachin H. Rohane

YSPM's, Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Dist. Satara, Maharashtra, India.

***Corresponding Author: Pratik R. Bhosale**

YSPM's, Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Dist. Satara, Maharashtra, India.

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ABSTRACT

Today's generation of university graduates will be required to continuously update existing employability skills and obtain new skills and qualifications as a result of the rapidly changing economy market. In order to engage in a multilateral discourse on employability skills between various educational stakeholders, it is important to consider the perceptions of both recent university graduates and faculty members. Often, recent university graduates do not fully possess the types and range of skills necessary for success in the workplace. Where such skills are taught, however, students often lack the awareness to articulate the types of skills learnt or developed in the classroom. Employability skills are best developed when these skills are integrated across the curriculum. Effective teaching practices such as promoting active learning, using multiple teaching strategies and providing prompt feedback all contribute to the skills development of students and recent university graduates. Results suggest that, whilst students would accept peer assessment as an element of their course, its introduction at least should focus on the development of evaluative skills and provide support to alleviate an onerous sense of responsibility. It is concluded that, if the value of peer assessment in terms of employability skill development is accepted, then it should be adopted as regular practice on undergraduate programmes wishing to equip students with a complete repertoire of employment-relevant skills. A systematic random sampling technique was used in selecting a sample of 392 Pharmacy graduate students drawn from different 6 institutes in Satara region from the academic session (2018-19).

KEYWORDS: Employability skills, Education, Knowledge.

INTRODUCTION

The landscape of graduate recruitment has changed drastically over the last few decades. The era where candidates were hired based solely on hard technical knowledge as reflected in academic qualifications or work experience has given way to a call for graduates who wield a formidable array of the softer, people-oriented, work-related skills. Graduates' employability is one of the fiercely debated issues in the current economic climate. Rapid changes taking place in the economy create a pressure upon employers to identify and recruit graduates that possess critical employability skills relevant to current demands. The emphasis being placed on these soft skills, also known as employability skills, is associated with and reflective of the current trends in graduate recruitment. Employers expect students to have well developed employability skills, so that they can make an immediate contribution to the workplace when recruited. Employability skills term varies by country. Another term used for Employability skills were the soft skills, generic skills, core skills or essential skills.[1]

The best results seem to be achieved when employability skill training is integrated with academic and vocational skill training forming a set of five basic skills. In this way, the relevance of the five types of skills are interrelated and taught as basic to job market success something in which the learner has a level of interest. The following strategies are suggested for incorporating employability skill development concepts in the classroom.[2]

Shivpuri & Kim, pointed that employers want to hire students with the appropriate skill set for the job. These skills include communication, problem-solving, and teamwork skills. Employability skills studied were problem-solving skills, communication skills, teamwork skills, change and innovation behavior, ability to manage self, and being civic-minded.[3]

The demand for graduates to use their subject knowledge in subsequent employment is minimal, but the opportunity to utilize their employability skills is tremendous. They have suggested that graduates must not only be able to access information, but apply the information through problem solving and teamwork processes.[4]

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Based their study on employability skills: communication, team-working, problem solving, literacy, numeracy, general information technology (IT), timekeeping, business awareness, customer-care, personal presentation, enthusiasm/commitment, enterprising, vocational job-seeking and advanced vocational job specific skills.[5]

Overtoom, proposed that employability skills are those basic skills necessary for getting, keeping, and doing well into job. It is a group of important skills instilled in each individual in order to produce productive workforce. This is parallel with individuals who have strong characteristics such as a high sense of self, innovative, productive, skilful, and competitive, a strong sense of determination, and creative in facing the challenges of the national as well as global platforms. Besides that, employability skill is also crucial in all professions as well as in education. The Conference Board 1996 defined employability skills as individual quality required by the employer which can be applied in various fields of work.[6]

Khaled Nordin proposed that changes in the industrial sector require educational institutions to provide graduates with employability skills Technical and vocational education systems need to plan strategies to improve the quality of graduates in order to meet the current needs of employers.[7]

The two greatest concerns of employers/recruiters today are finding good employees and training them. It is observed that there lies a difference between the skills needed on the job and those possessed by applicants called the skills-gap, which is of real concern to human resource managers and business owners looking to hire competent skillful employees. While employers would prefer to hire people who are trained and ready to go to work, they are usually willing to provide the specialized, job specific training necessary for those lacking such skills. Employability has become a far bigger challenge than unemployment, probably because there are still large vacancies in industries but lack of employable candidates.[8]

The technical and management education system in the country has grown extremely. While on the one side we say that we have the world‘s largest stock of engineers, scientists and management graduates, we have not been able to derive full economic benefit from this talent base probably because of the mismatch between industrial needs and educational output. It also seems that students often undervalue the need to possess transferable skills. Instead, they deem that mastery of disciplinary content is more important than transferable skills to employers. Though, employers desire graduates who can think and work on their feet and determine ways to accomplish tasks.[9]

Today, employers in every industrial sector stress the need for employees with certain set of foundational skills. These include a strong academic grounding along with individual abilities such as teamwork, problem solving, work ethic and integrity. While employers rely on employees to have the same basic skills, they do not always talk about or label them the same way. This makes it difficult for prospective employees and educators to know exactly what it takes to be ready to succeed in any career path in any industry.[10] Employability skills are therefore valued as they apply to many jobs and so can support common preparation to meet the needs of many different professions.[11]

It also refers to those skills required to acquire and retain a job. These transferable skills include the ability to solve complex multidisciplinary problems, work successfully in teams, exhibit effective oral and written communication skills, and practice good interpersonal skills.[12]

The lack of adequate skills and high attrition rates has a huge impact in terms of India‘s ability to absorb new technologies and new solutions. So there seems to be need for a fundamental shift toward an emphasis on general skills in education because the skills most in demand are least in supply.[13]

In recent practice today this term employability skills is often used to describe the preparation skills upon which an applicant student must build job specific skills which relate to communication, personal and interpersonal relationships, problem solving, and management of organizational processes.[14]

The National Association of College and Employers 2014, have compiled a list of the top 18 skills requested by employers. These skills in rank order are as follows: (1) Communication skills (2) Leadership (3) Analytical/quantitative skills; (4) Strong work ethic (5) Teamwork skills (6) Problem solving skills (7) Initiative (8) Detail-oriented (9) Computer skills (10) Technical skills (11) Flexibility/adaptability (12) Interpersonal skills (13) Organizational ability (14) Strategic planning skills (15) Friendly/outgoing personality (16) Entrepreneurial skills/risk-taker (17) Tactfulness and (18) Creativity. These skills are considered to be important for potential employees to possess and apply to their job.[15]

Purpose of study

1. To identify the new ways to improve the skills in the course of higher education
2. To assess skill gap of management students that are developed in academic with regards to the level of expectation of the industry.
3. To identify the employability skills, which are classified into three groups - Person to Person, Person to Vocation and Person to Job.

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Significance of the Study

The findings will enable the students to take necessary actions to plan their career and know and reinforce the employability skills. Similarly the institutes will be able to plan for value addition to their students to make them more employable. The results of this research work will no doubt be of great importance to all stakeholders that includes the researchers, students, Institutes and employers. This study will equally form useful reference materials to both the researchers and students.

METHODOLOGY

This study used a descriptive research design with quantitative approached. This study aims to identify the importance of employability skills. Survey research design method was used for the study, which involves the collection of information from a sample of individuals through their responses to predetermined questions. Survey design was chosen for this study as it is supposed to be the most appropriate for gathering first hand information on students employability skills and opportunities, without changing or modifying the situation under investigation. No cause-and-effect relationship was sought.

A systematic random sampling technique was used in selecting a sample of 392 Pharmacy graduate students drawn from different 6 institutes in Satara region from the academic session (2018-19). A structured questionnaire titled study on Pharmacy Graduate students Employability was used for collection of data. The test-retest method of reliability was used.

RESULTS

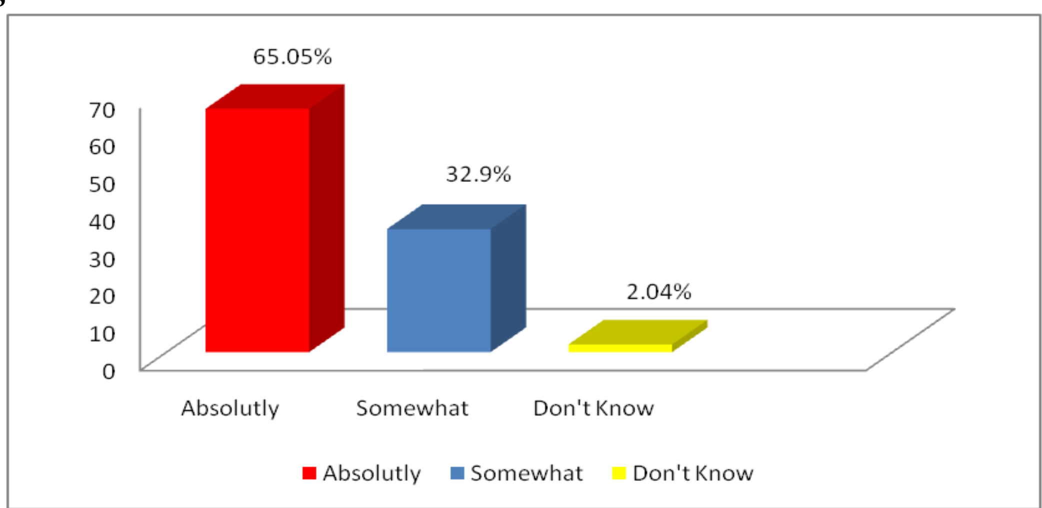

Figure 1: Career Objective.

Figure 1:- The survey conducted for the above study revealed that almost 65.05% candidates were absolutely aware about their own career, whereas around 32.9% were not much clear about their own career objectives and 2.04% were not at all aware about their own career objective.

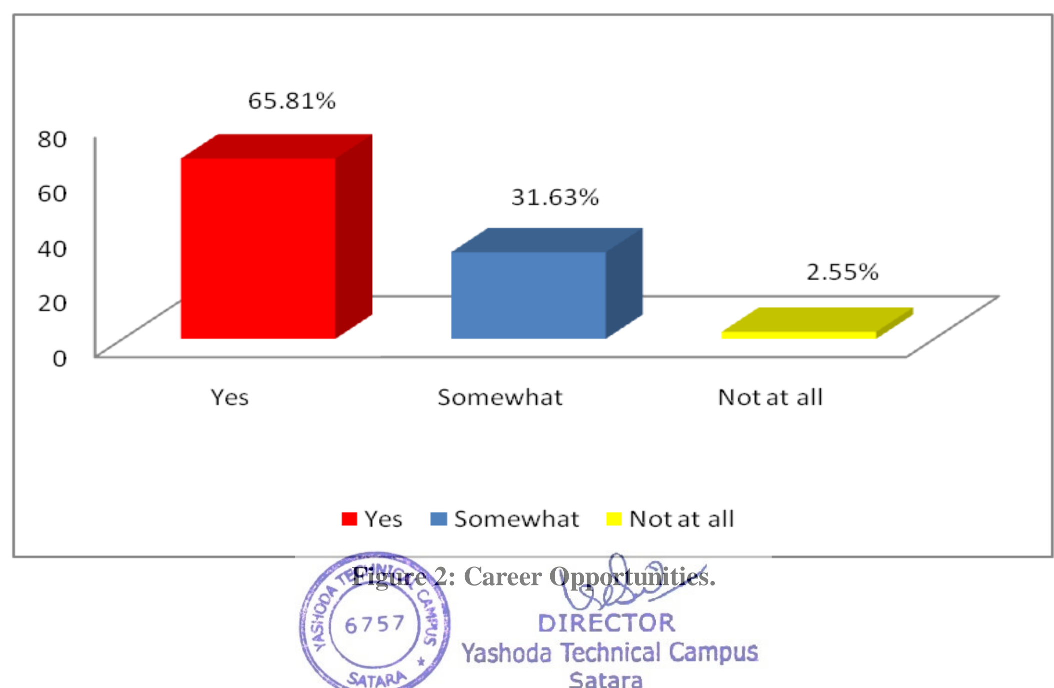

Figure 2: Career Opportunities.

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Figure 2:- Depicts that 65.81% respondents were aware about the career opportunities available in the field, but nearly 31.63% were not fully aware about such opportunities 2.55% were not at all aware about their own career opportunities.

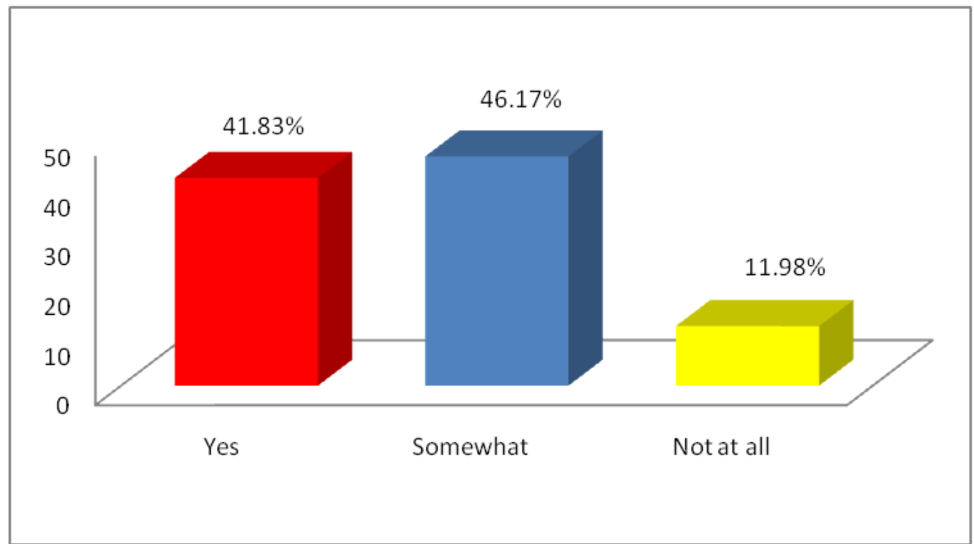

Figure 3: Course content Updation.

Figure 3:- reveals that only 41.83% respondents think that their course content is updated with the current industrial requirement whereas around 46.17% candidates believe that their syllabus is not completely updated with the current industrial requirement and 11.98% believe that it is not at all updated with current industrial requirement. It has also been found that some private universities who update their syllabus regularly do not face such problem and that increases the chances of opportunity for their students.

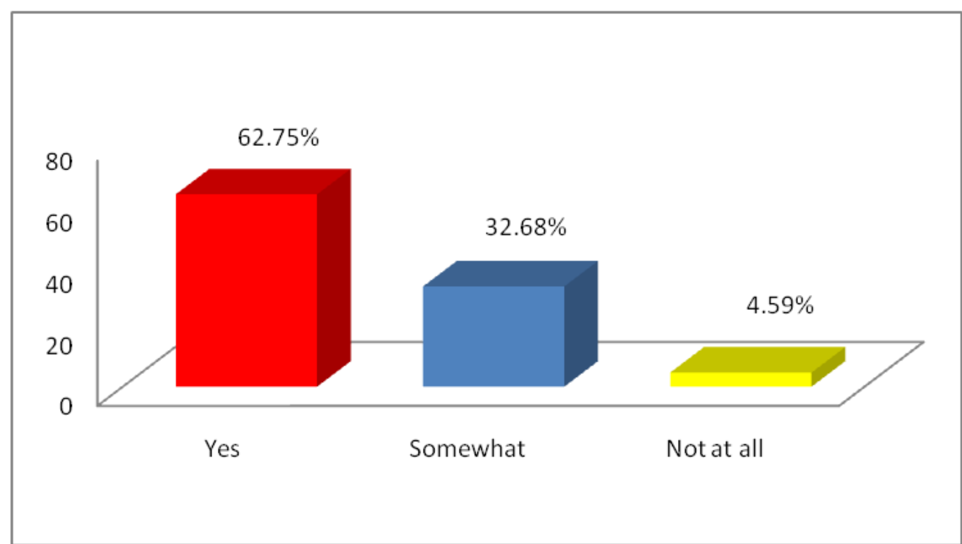

Figure 4: Advantage of Extra Curricular.

Figure 4:- Depicts that 62.75% respondents believed that participation in extracurricular activities during college definitely/surely adds an advantage during recruitment process whereas 32.68% think that it is less advantageous and 4.59% were not at all advantagous about extra-curricular.

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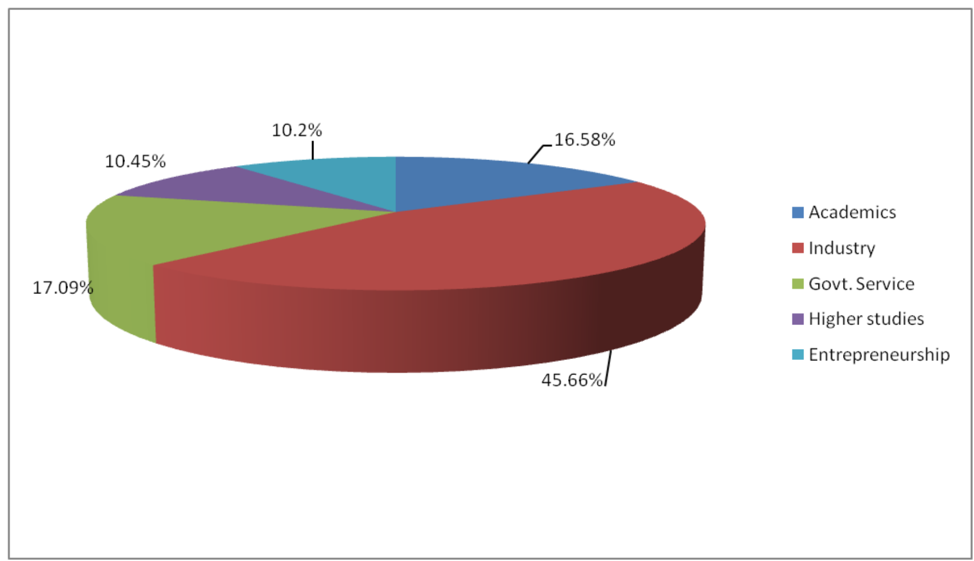

Figure 5: Career preference.

Figure 5:- Reveals that 45.66% of the candidates preferred to make their career in industry followed by government services 17.09% and few preferred to join academics 16.98%. And very few preferred to go for higher studies and start their own business as an entrepreneur. This shows that majority of population is interested to do their career in industry where as they are not equipped with the current industrial requirement could be one of the reason for unemployment.

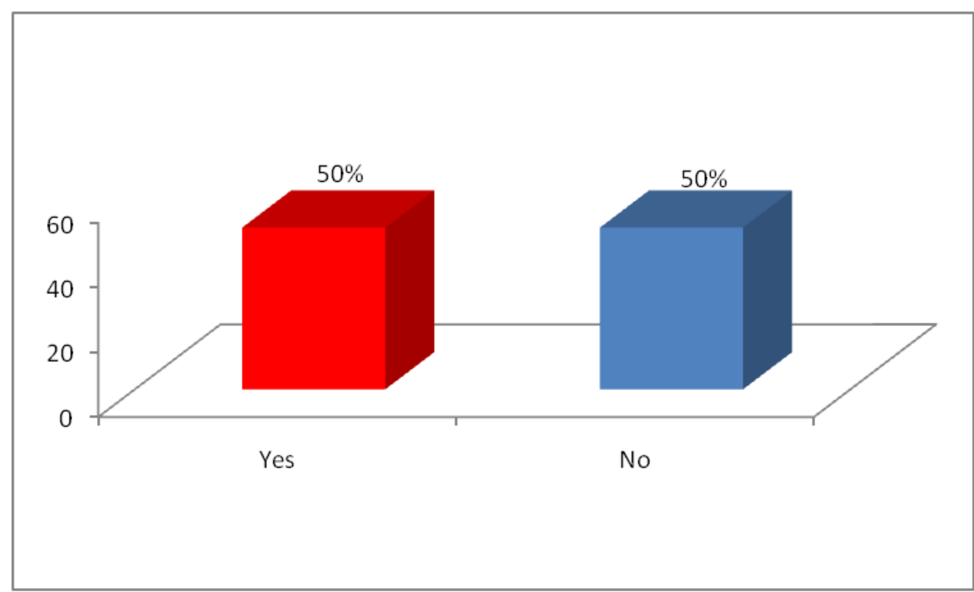

Figure 6: Sufficiency of Possessing only Degree.

Figure 6:- Reveals that 50% of candidates strongly believe that only possessing a degree is not sufficient for securing good opportunity in the industry and believed that some additional skills are surely required along with the degree to convert a good opportunity into a job. But most of the candidates were not sure about the exact set of additional skills required for the same.

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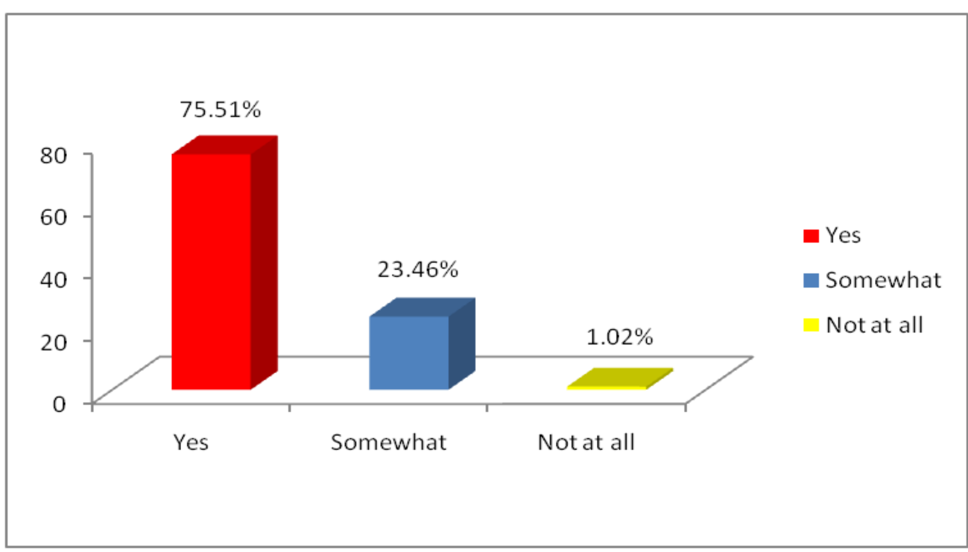

Figure 7: Acquiring Additional Skills.

Figure 7:- Depicts that around 75.51% respondents agreed to and tried to acquire such additional skills which they think is essential, whereas remaining 23.46% tried something for skills and there is 1.02% are not at all seriously to acquire such additional skills.

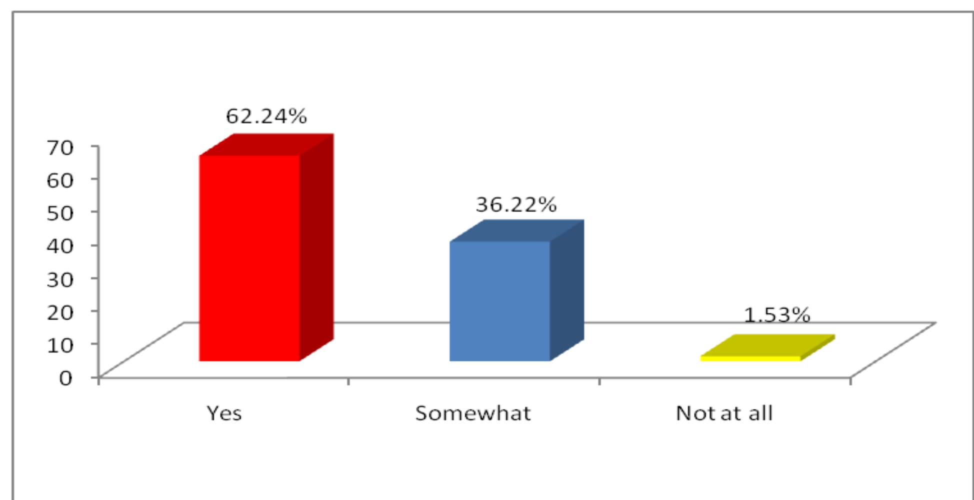

Figure 8: Awareness about Employability Skills.

Figure 8 reveals that only 62.24% respondents were well aware about the employability skills whereas 36.22% were little aware and 1.53% were totally unaware about the concept of employability skills. But when they were made aware about the concept of employability skills, more than 95% agreed that these skills are most and absolutely essential for securing a good job in the today‘s competitive world.

Table 1: Responses for the different employability skills.

Sr. No.	Employability skills	Excellent (%)	Good (%)	Poor (%)
1	Communication Skills	46.60	54.84	2.55
2	Teamwork Skills	42.34	56.63	1.02
3	Problem Solving Skills	35.96	59.18	2.55
4	Initiative & Enterprise Skills	31.12	65.30	3.57
5	Planning & Organizing Skills	36.22	60.20	1.02
6	Learning Skills	39.79	53.06	2.29
7	Technology Skills	33.92	54.84	2.55
8	Self Management Skills	40.56	56.63	2.80
9	Personal Attribute skills	43.11	54.08	2.80

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Table 1:- The responses of the candidates regarding awareness about nine different employability skills identified. It reveals that majority of these skills, the candidates were only aware of and therefore required to analyze and acquire such skills to increase their employability. Out of the nine set of skills identified students have mainly concentrated on communication and technology skills and less on the other skills which might be the reason for generation of qualified but less skillful candidates required today by the corporate.

Table 2: Responses for the different activities undertaken to increase employability skills.

Sr. No.	Activities	Yes (%)	No (%)
1	Prepared/updated my CV	71.42	28.57
2	Sought advice on how to write a CV	67.34	32.65
3	Found out what skills/qualities employers are looking for	76.53	23.46
4	Kept record of my personal development and achievements	77.04	22.95
5	Did some research on careers I am interested in	72.19	27.80
6	Visited a careers/job fairs	65.81	34.18
7	Undergone any careers module/course	70.66	29.33
8	Attended any Short term courses/ seminars/conference	75	25
9	Worked on enhancing communication skills	75	25
10	Tried to communicate with employers by sending resumes	59.69	40.30
11	Worked part time to get additional experience	58.92	41.07
12	Took advise of counselor	67.85	32.14
13	Used internet to get important information of the field	100	0

Table 2:- The responses of the candidates regarding the various activities undertaken by them to enhance their awareness to increase their chances of employment. It reveals that most of the identified activities were undertaken by the students (some knowingly and some unknowingly) but with a less serious note probably due to lack of awareness about existence of such employability skills and therefore it is required to be taken seriously to make them more employable.

CONCLUSION

The concept of employability skills has increasingly become the concern of stakeholders like industries, employers, education institutes and indeed almost all the students. It largely includes student's skills and potentials for obtaining and succeeding in a job apart from their routine academic knowledge and skills. From the present examination we can along these lines presume that just specialized information (Degree) isn't adequate for verifying a great job, yet understudies should focus on increasing the value of their profile as some arrangement of aptitudes currently called as employability abilities required by the businesses in their field to make them progressively employable. The study also concludes that in the competitive age students should voluntarily try to identify and acquire these skills along with their course to make them more employable. The institutes should also create an environment that will assist the students to boost their employability skills by conducting mocks, seminars, workshops, in-house skill improvement programme etc. With Industry's increasing demand for skilled rather than qualified talent; it is important to understand the needs of demand side better and take combined efforts by all the stakeholders at large.

This investigation has featured the significance of employability abilities of the understudies for better business opportunity. It will likewise help in distinguishing the difficulties that should be survived if more understudies are to grasp the potential advantages by securing these aptitudes.

So it could be recommended that students during their graduation should try to identify the employability skills required in the pharmaceutical field or their field of interest and work on it to make themselves potent employable candidates. Understudies likewise do some specialized extra course to improve their particular aptitudes sets.

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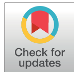
Citric acid crosslinked carboxymethylcellulose-polyvinyl alcohol hydrogel films for extended release of water soluble basic drugs



Vishwajeet Sampatrao Ghorpade[a,*], Remeth Jacky Dias[b], Kailas Krishnat Mali[a], Safina Ismail Mulla[a]

[a] *Department of Pharmaceutics, YSPM's Yashoda Technical Campus, Wadhephata, Satara, 415011, Maharashtra, India*
[b] *Department of Pharmacy, Government Polytechnic, Jalgaon, Maharashtra, India*

A R T I C L E I N F O

Keywords:
Carboxymethylcellulose
Polyvinyl alcohol
Hydrogel films
Extended release
Basic drugs
Gentamicin

A B S T R A C T

The aim of present work was to develop carboxymethylcellulose (CMC)-polyvinyl alcohol (PVA) hydrogel films for extended delivery of water soluble basic drug, using citric acid (CA) as a cheap and non-toxic crosslinking agent. Gentamicin sulfate (GTM) was used as a model drug. The hydrogel films were evaluated for carboxyl content, tensile strength, swellability, drug loading and release, hemocompatibility and characterized by ^{13}C-CP-MAS NMR, ATR-FTIR and thermal analysis (TGA and DSC). The instrumental analysis helped to confirm the formation of ester crosslinks. CMC-PVA hydrogel films exhibited greater carboxyl content, tensile strength and swellability than the pure CMC hydrogel films. The GTM loading increased with an increase in the amount of PVA in the hydrogel films. The CMC-PVA hydrogel films showed propensity to extend the release of GTM above 24 h. Hemolysis assay revealed the hemocompatible nature of the hydrogel films. Altogether, the CMC-PVA hydrogel films can be envisioned as promising biomaterial for the delivery of water soluble basic drugs.

1. Introduction

Hydrogels are the crosslinked polymeric network structures capable of absorbing large amount of water without dissolving in it. Due to their rubbery consistency and water retention capacity which resembles natural tissues, hydrogels are mostly used in the field of biomedical and pharmaceutical sciences [1]. The hydrogels can be prepared from synthetic or natural polymers using physical, chemical or radiation crosslinking. The hydrogels prepared using synthetic polymers exhibit good mechanical properties; however, most of them do not support cell adhesion and tissue formation. On the other side, natural polymer based hydrogels are biocompatible, biodegradable and support cellular activities [2]. In recent years, extensive research has been carried out to develop hydrogel-based drug delivery systems using natural polymers [3–9]. Amongst the natural and semi-synthetic polymers, cellulose and its water soluble derivatives are mostly preferred because of the abundance of cellulose on earth and relatively low cost of the cellulose derivatives than the synthetic polymers [10].

Carboxymethylcellulose (CMC) is highly hydrophilic cellulose derivate [11,12]. It is widely used as thickening and suspending agent in the food and pharmaceutical industry. The scientists dealing with the hydrogels for drug delivery are interested in CMC due to its good swellability, non-toxicity and modifiability [13]. Large number of reports are available on the preparation of CMC-based hydrogels for the delivery of water soluble drugs [14–17]. In most of these cases, the crosslinking agents used are either expensive or toxic [18]. In last few years, citric acid (CA) has emerged as a non-toxic and cheap crosslinking agent. At high temperatures, it esterifies the hydroxyl groups present on the nearby polymer chains and forms the crosslink. For preparation of CA crosslinked hydrogels of alone CMC, excess of CA is required as CMC shows poor crosslinking due to electrostatic repulsion in between the carboxylate ions present on the adjacent polymer chains. This leads to the formation of intramolecular crosslinks. Therefore, Demitri and co-workers (2008) fabricated CA crosslinked superabsorbent hydrogels by combining CMC with hydroxyethylcellulose (HEC) for improving the intermolecular crosslinking [19]. These hydrogels were intended for agricultural application. The hydroxyl groups attached to the oxyethylene chains in HEC are highly reactive and hence, can readily undergo esterification reaction with CA and facilitate the formation of CMC hydrogels with good integrity. Very few works are reported on the use of CA crosslinked CMC based hydrogels in drug delivery. Mali et al. (2018) have prepared hydrogel films comprised of CMC and tamarind gum (TG) for delivery of moxifloxacin HCl. Furthermore, we have prepared CMC-polyethylene glycol

* Corresponding author.
E-mail address: vsg.bpharm@yes.edu.in (V.S. Ghorpade).

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(PEG) hydrogel films for the improvement of loading and extended release of hydrophobic drugs [20].

Polyvinylalcohol (PVA) is a synthetic polymer which is biocompatible, biodegradable and widely used in pharmaceutical, bio-medical and food sectors [21]. The large number of reactive hydroxyl groups present on the polymer chain of PVA makes it suitable for the synthesis of hydrogels, mostly by physical [22–24] or chemical [25–28] crosslinking. The CA crosslinked PVA films have been prepared for antimicrobial packaging purpose [29]. Taking this into consideration, PVA can be used as a better alternative to HEC while preparing CMC hydrogels since the amount of PVA required for the formation of intact hydrogels will be less. In previous studies, CMC-PVA hydrogels have been prepared for drug delivery [30–32]; however, CA crosslinked CMC-PVA hydrogels have not been investigated for their ability to control the drug release.

The present work deals with the preparation of CMC-PVA hydrogel films using CA as crosslinking agent, for the delivery of water soluble basic drugs. Our objective was to develop the inexpensive hydrogels with good drug loading efficiency and ability to control the release of water soluble drugs, so that they can be used as implants or wound dressings. As the crosslinks consist of COOH branching which usually ionize at physiological pH, the prepared hydrogel films could be more favorable for the delivery of basic drugs. Along with an improvement in the extent of crosslinking, the large number of OH groups on PVA chains may also contribute towards the enhancement of swellability of the CMC-PVA hydrogel films. Also, the OH groups of PVA and COOH groups of CA crosslinks may hold the weakly basic drug molecules (pKa > physiological pH) within the hydrogel matrix by hydrogen bonding and electrostatic interactions. Taking these aspects into account, we hypothesize that the CA crosslinked CMC-PVA hydrogel films may enhance the loading as well as control the release of water soluble basic drug.

The hydrogel films were suitably characterized to confirm the formation of ester crosslinks. The films were loaded with gentamicin sulfate (GTM, model water soluble basic drug) and evaluated for drug release. Hemolysis assay was performed to test the hemocompatibility of the hydrogel films.

2. Materials and methods

2.1. Materials

Gentamicin sulfate (GTM) was obtained as a gift sample from RP Pharma, Mumbai, Maharashtra (India), sodium carboxymethylcellulose (CMC, degree of substitution: 0.7, average molecular weight: ~250000) was purchased from Sigma Aldrich, Mumbai, Maharashtra (India), polyvinyl alcohol (PVA, MW: 115,000 Da, Hydrolysis: 98–99%) and citric acid (CA) were purchased from Loba Chemie, Mumbai, Maharashtra (India). All other chemicals were of analytical grade and used as received.

2.2. Preparation of CMC-PVA hydrogel films

The CMC-PVA hydrogel films were prepared by modifying the previously reported method [20]. Initially, a suitable amount of PVA was dissolved in distilled water by heating on a water bath at 80 °C for 20 min, under reflux. The obtained PVA solution was cooled to room temperature followed by addition of CMC and CA (% of polymer amount). This mixture was stirred on a magnetic stirrer to obtain a homogenous solution and placed overnight in a closed compartment in order to remove the air bubbles. The clear solution was poured into the petri dish of specific diameter (9 cm) and dried in the hot air oven at 50 °C for 24 h. The dried CMC-PVA-CA film was cured at 145 °C for 5 min, to promote the crosslinking reaction in between the polymer chains. The cured film was washed with distilled water in cycles until the pH of water became nearly neutral. Finally, the swollen film was washed with isopropyl alcohol to remove the water entrapped within the matrix. The shrunken film so obtained was dried in the hot air oven at 40 °C for 24 h and stored in a desiccator. The concentration of PVA was varied in order to study their effect on the film properties (see Table 1).

Table 1
CMC-PVA hydrogel films containing different concentrations of PVA.

Batch	CMC (%w/v)	PVA (%w/v)	CA (%)[a]
HF0	2	0	20
HF0.5	2	0.1	20
HF1	2	0.2	20
HF2	2	0.4	20
HF3	2	0.6	20

[a] Indicates % of the polymer amount.

2.3. Determination of carboxyl content

The total carboxyl content of hydrogel films was determined by acid-base titration [33,34]. CO_2 free water was used for the preparation of titrant (0.1 N HCl) and analyte (0.1 N NaOH). A specific amount of hydrogel film was added to the adequate volume of 0.1 N NaOH and stirred on a magnetic stirrer for 2 h. The hydrogel films disintegrated and dissolved completely due to sodium hydroxide aided breaking of the ester crosslinks. Also, sodium hydroxide reacts with the free carboxylic acid groups to form sodium citrate. The excess amount of 0.1 N NaOH was titrated with 0.1 N HC1 using phenolphthalein indicator. The carboxyl content in milliequivalents per 100 g of hydrogel film was determined using following formula:

$$Total\ carboxyl\ content\ (mEq/100g) = \frac{(V_b - V_a) \times N_{HCl} \times 100}{W_{HF}} \quad (1)$$

where, N_{HCl} is the normality of HCl (mEq/mL), V_b and V_a are the volumes of HCl in absence and presence of hydrogel film, and W_{HF} is the weight of hydrogel sample (g).

2.4. Characterization of hydrogel films

Solid state ^{13}C cross-polarization-magic angle spinning (^{13}C CP-MAS) NMR spectra of CMC, PVA, CA and CMC-PVA hydrogel film (HF3) was recorded using JEOL-ECX400 spectrometer operated at 400 MHz, with a spinning speed of 10 KHz. The contact time, relaxation delay and sweep width were fixed at 3.5 ms, 5 s and 35 kHz, respectively. The external hexamethylbenzene standard methyl resonance at 17.3 ppm was used for the calibration of chemical shifts.

The infrared spectra of CMC, PVA, CA and hydrogel films were obtained using ATR-FTIR spectrophotometer (MIRacle-10, IR Affinity-1, Shimadzu, Japan). The samples were transferred to the ATR compartment and spectra were obtained in the range of 400–4000 cm^{-1} (scans = 25; resolution = 4 cm^{-1}).

CMC, PVA and hydrogel films (HF0 and HF3) were subjected to the thermal analysis (TGA and DSC) using SDT Q600 V20.9 Build 20 (TA instruments, Water, USA). Samples (approx. 5 mg) were taken in the sample pan and were heated from 30°C to 300 °C, at the heating rate of 10 °C/min, under nitrogen atmosphere (purge rate = 10 mL/min).

2.5. Determination of tensile strength

The tensile strength of the hydrogel films was tested using Texture Analyzer (CT 3-10,000, Brookfield, WI) [35,36]. The analyzer was fitted with a 10 kg load cell. Each film was cut into 2 cm × 1 cm size and clamped on probe TA-DGA (hold time = 60 s). Holding the lower clamp stationary, the hydrogel film was pulled apart with the help of upper clamp with a speed of 2 mm/s to a distance of 6 mm (trigger load = 0.05 N). The force at which the film broke was recorded and the

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tensile strength of the hydrogel films was determined as given below.

$$Tensile\ strength\ (kg/mm^2) = \frac{Force\ at\ break}{Initial\ cross-sectional\ area} \quad (2)$$

2.6. Determination of swelling behavior

The swelling behavior of the hydrogel films was studied in phosphate buffer (pH 7.4) at 37 °C. The hydrogel films (~0.2 g) were immersed in the beaker containing 20 mL buffer. After 30 min, swollen film samples were removed from the buffer and their surface was blotted with tissue paper to remove the excess water followed by weighing on the analytical balance (Shimadzu, AX 120). The hydrogel films were reimmersed in the buffer and the procedure was repeated at specific time intervals till 24 h. The swelling ratio of the hydrogel films was determined by using eq. no. (3).

$$Swelling\ ratio\ (g/g) = \frac{W_s - W_d}{W_d} \quad (3)$$

where, W_s is the weight of swollen hydrogel film and W_d is the weight of dry hydrogel film. The measurements were taken in triplicate [3,37].

2.7. GTM loading

In order to load GTM into the hydrogel films, the preweighed films (~100 mg) were allowed to swell in the aqueous GTM solution (10 mg/mL) till equilibrium. The swollen hydrogel films were dried at room temperature for 24 h. In order to determine the amount of GTM loaded in the hydrogel films, they were initially cut into fine pieces. The specific amount of these pieces were added to 50 mL phosphate buffer (pH 7.4) and stirred for 48 h. Thereafter, the amount of GTM released in the buffer was determined using colorimetric estimation as GTM shows negligible UV absorbance [38]. Briefly, standard solutions of GTM (5–9 μg/mL) were prepared in phosphate buffer (pH: 7.4). One mL of these solutions was taken in the 10 mL volumetric flasks followed by addition of 0.5 mL of 2 M H_2SO_4 and 2 mL of 5×10^{-4} M $KMnO_4$ solutions. The obtained mixtures in the flasks were heated on the water bath for 25 min, cooled to room temperature and 2.5 mL of 5×10^{-4} M methylene blue was added to the respective flasks. The volume was adjusted to 10 mL with buffer and the mixtures were subjected to spectrophotometric analysis (UV1800, Shimadzu, Japan) at 664 nm. The calibration curve so obtained was used further for determination of GTM released in the buffer [39].

2.8. In vitro GTM release

The in vitro drug release study was performed by modifying the previously reported methods [40,41]. The GTM loaded hydrogel films of known weight were placed in screw-capped vials containing 10 mL of phosphate buffer (pH: 7.4). These vials were kept in the constant temperature bath (without agitation) at 37 °C. At specific time intervals, aliquots were withdrawn and suitably diluted followed by spectrophotometric analysis (UV1800, Shimadzu, Japan) using colorimetric method as described above. All the measurements were taken in triplicate.

2.9. Hemocompatibility study

Recently, biocompatibility of natural polymer based biomaterials was tested by few authors using hemolysis assay [5,7,9]. As the CMC-PVA hydrogel films can be used as implants or wound dressings, they were examined for hemocompatibility using hemolysis assay with slight modification [42]. The hydrogel films were cut in order to get surface area of 2 cm^2. These films were allowed to swell in the phosphate buffer saline (PBS) maintained at 37 °C for 1 h. Thereafter, PBS was removed and 0.5 mL of human CPD (citrate-phosphate-dextrose) blood was added over the films. After 20 min, the hemolysis process was stopped by addition of 4 mL, 0.9% NaCl saline followed by incubation of the samples for 1 h at 37 °C. The incubated samples were centrifuged at 4000 rpm for 10 min and the supernatant was subjected to spectrophotometric analysis (UV1800, Shimadzu, Japan) at 545 nm. The hemolysis (%) was determined using following formula:

$$Hemolysis\ (\%) = \left(\frac{A_{Test\ sample} - A_{-ve\ control}}{A_{+ve\ control} - A_{-ve\ control}}\right) \times 100 \quad (5)$$

where, A = absorbance.

+ve control = mixture of 0.5 mL, human CPD blood and 4 mL double distilled water

- ve control = mixture of 0.5 mL, 0.9% NaCl saline and 4 mL double distilled water

2.10. Statistical analysis

The experimental data were compared by one-way analysis of variance (ANOVA) using GraphPad Prism 5.01 software. The statistical significance was considered at $p < 0.05$.

3. Results and discussion

3.1. Formation of CMC-PVA hydrogel films

The CMC-PVA hydrogel films were obtained by esterification-crosslinking mechanism. At high temperatures, CA gets converted into an anhydride which esterifies the OH groups of CMC and PVA. This leads to the formation of ester crosslinks in between the polymer chains. Fig. 1 displays the possible reaction involved in the formation of CMC-PVA hydrogel films. Besides CMC-PVA crosslinks, there may be presence of CMC-CMC and PVA-PVA crosslinks in the hydrogel matrix. The concentration of CA, curing temperature and curing time required for the formation of the hydrogel films was decided on the basis of preliminary studies. The minimum concentration of CA needed for the formation of hydrogel film with good matrix integrity was 20%. On increasing the CA concentration above 20%, the hydrogels exhibited poor swellability. Although the previous reports indicate the formation of hydroxyl acid crosslinked cellulose-based hydrogels at the curing temperature of 80 °C, the time required for the curing was more (> 10 h) [19,43]. In the preliminary studies, we found that the curing temperature of 145 °C and curing time of 5 min was sufficient to obtain the hydrogel films with desired swellability and integrity. However, increasing the curing temperature and curing time above 145 °C and 5 min led to the development of yellowish color on the films, possibly due to thermal decomposition. The obtained hydrogel films showed an average thickness in the range of ~130–135 μm.

3.2. Total carboxyl content

Table 2 represents the total carboxyl content of the CMC-PVA hydrogel films. It was observed that an increase in the amount of PVA up to 0.2% increased the carboxyl content of the hydrogel films. In case of CMC hydrogel film (HF0), the carboxyl content was found to be less as compared to the CMC-PVA hydrogel films. This indicates that CMC hydrogel film showed minimum number of interpolymer crosslinks than the CMC-PVA hydrogel films. This can be attributed to the degree of substitution (DS) of CMC i.e. 0.7 and weak electrostatic repulsion in between the adjacent CMC chains due to presence of few COO^- groups in the feed of HF0. The DS equivalent to 0.7 suggests that very few primary hydroxyl groups (C6-OH) are available on CMC for taking part in the esterification reaction. On the other hand, the weak electrostatic repulsion in between the adjacent CMC chains may inhibit the CA from linking them. As a result, most of the CA may remain unutilized. PVA

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Fig. 1. Reaction involved in the formation of CMC-PVA hydrogel films.

consists of large number of secondary hydroxyl groups which are highly reactive [44]. These hydroxyl groups can readily participate in the esterification reaction. On inclusion of PVA in the feed, the CA which was unutilized in case of CMC hydrogels will get involved in the formation of CMC-PVA and PVA-PVA crosslinks. This may be the reason behind increase in the carboxyl content of HF0.5 and HF1. However, further increase in the amount of PVA (HF2 and HF3) led to decrease in the carboxyl content possibly due to decrease in the crosslinking density of the hydrogel films caused by dilution effect. In case of batch HF1 (0.2% PVA), there might be a possibility of maximum consumption of the citric acid in the formation of crosslinks. As a result, further increase in the concentration of PVA may increase the distance in between the adjacent crosslinks within the hydrogel films and reduce the crosslinking density. Another reason behind decrease in the crosslinking density on increasing the concentration of PVA beyond 0.2% may be insufficient physical interactions in between CMC and PVA at high concentrations of PVA. HF3 containing high amount of PVA showed low carboxyl content than HF1 and HF2 but it was greater than HF0.

Table 2
Total carboxyl content (TCC), tensile strength (TS), equilibrium swelling ratio (ESR), GTM loading and hemolytic activity of the CMC-PVA hydrogel films.

Batch	TCC (mEq/100 g)	TS (kg/mm^2)	ESR (g/g)	GTM loading[a]	Hemolysis (%)
HF0	229.55	3.13 ± 0.16	50.51 ± 3.63	137.51 ± 1.56	4.15 ± 0.22
HF0.5	391.20	3.38 ± 0.09	53.55 ± 2.75	162.24 ± 2.12	4.52 ± 0.83
HF1	417.35	3.54 ± 0.12	59.75 ± 1.90	190.40 ± 1.04	4.02 ± 0.71
HF2	372.81	3.49 ± 0.05	66.42 ± 1.88	204.22 ± 2.13	3.87 ± 0.54
HF3	354.91	3.45 ± 0.13	77.31 ± 2.96	216.03 ± 1.89	4.34 ± 0.48

[a] Indicates readings in mg/g of hydrogel films.

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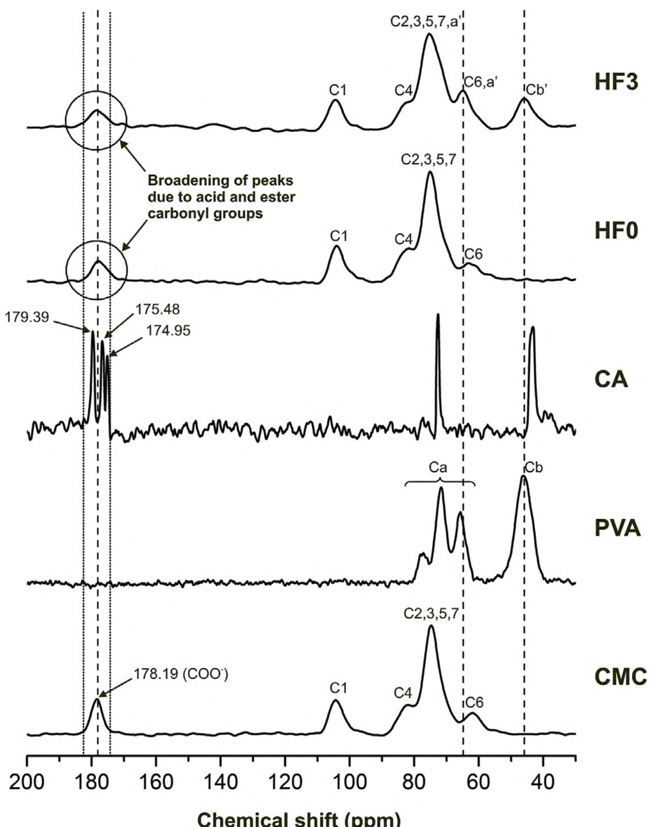

Fig. 2. Solid state ^{13}C-NMR of CMC, PVA, CA, HF0 and HF3.

3.3. Solid state ^{13}C-NMR analysis

The solid state ^{13}C-NMR spectrum of CMC, PVA, CA, CMC hydrogel film (HF0) and CMC-PVA hydrogel film (HF3) is shown in Fig. 2. The symbolic representation of various carbon atoms of CMC and PVA is indicated in Fig. 1. The carbon atoms of CMC showed a signals in the region 56-66(C6), 68-80(C2,3,5,7), 81-89(C4), 98-109(C1) and 175-183(COO$^-$) ppm. The resonance peaks corresponding to the carbon atoms of PVA appeared at 46.16 ppm (Cb) and in the range of 60–80 ppm (Ca). The carbonyl carbons of citric acid showed sharp signals at 174.95, 175.48 and 179.39 ppm. The spectrum of CMC-PVA hydrogel film (HF3) showed existence of new peaks at 45.90 and 64.70 ppm which represent the peaks of PVA (Cb at 46.16 ppm and Ca at 65.64 ppm). These peaks were absent in the spectrum of pure CMC and CMC hydrogel film (HF0). The remaining peaks of PVA were not visible in the spectrum of HF3 because they overlapped with the peaks of carbons belonging to the anhydroglucose units of CMC. Besides, the sharp carboxylate carbon peak of CMC at 178.19 ppm was found to be slightly broadened towards up-field region in the spectrum of HF0 and HF3. This clearly indicates the presence of ester crosslinks in the hydrogel films.

3.4. ATR-FTIR analysis

The presence of ester crosslinks in the CMC-PVA hydrogel films was further confirmed from ATR-FTIR analysis. Fig. 3a depicts the ATR-FTIR spectra of CMC, PVA and CA, whereas spectra of hydrogel films (HF0-HF3) are presented in Fig. 3b. The spectrum of CMC showed the characteristics peaks at 3450 cm^{-1} (O-H stretching), 2920.52 cm^{-1} (C-H stretching), 1584 cm^{-1} (C=O stretching) and 1051 cm^{-1} (C-O stretching). In the spectrum of PVA, broad band was observed at 3257 cm^{-1} corresponding to O-H stretching and sharp peaks at 2929 cm^{-1} represented C-H stretching. The peaks of PVA at 1427 cm^{-1}, 1327 cm^{-1} and 839 cm^{-1} were related to the C-H bending whereas the prominent peak at 1082 cm^{-1} represented C-O stretching. The distinct IR peaks of CA were observed at 3498 cm^{-1} and 3283 cm^{-1} (O-H stretching), 1693 cm^{-1} (C=O stretching), 1199 cm^{-1} (C-O stretching) and 786 cm^{-1} (C-C stretching).

The spectrum of HF0 showed peak at 1722 cm^{-1} corresponding to the ester as well as carboxylic acid carbonyl stretching. This indicates the presence of ester crosslinks in the CMC hydrogel film (see Fig. 3b). The changes in the carbonyl peak of the hydrogel films on addition of PVA can be clearly observed in Fig. 3c. The intensity of this peak was found to be increased as we move from HF0 to HF1, which suggests an increase in the carboxyl content and hence, the extent of crosslinking of the hydrogel films as the concentration of PVA is increased up to 0.2%. As the concentration of PVA was further increased (HF2 and HF3), the carbonyl peak intensity was found to be reduced. This clearly reveals that increase in the concentration of PVA reduces the crosslinking density. The results of carboxyl content determination were in accordance with this finding. It was also noticed that the carbonyl peak shifted towards lower wavenumber with increase in the amount of PVA from 0.2% to 0.6% (HF1-HF3). An increase in the hydrogen bonding interaction in between the OH groups of PVA and carbonyl groups of free acid and ester groups of the CMC-PVA hydrogel films may be responsible for this shifting. The intensity of peak at 2942 cm^{-1} was found to be increased from HF0 to HF3 (see Fig. 3b) which can be attributed to an increase in the number of CH_2 groups associated with PVA.

3.5. Thermal analysis

Fig. 4 depicts the TGA, DTG and DSC profiles of CMC, PVA, HF0 and HF3. The TGA thermogram of CMC showed two stages whereas that of PVA showed three stages of mass loss (see Fig. 4a). In case of CMC, 10.56% mass loss occurred in between 40 °C to 163 °C due to loss of moisture followed by 42.19% mass loss in the range of 253 °C–316 °C, owing to thermal decomposition of the sample. The maximum degradation temperature (T_m) of CMC was observed at 295.11 °C (see Fig. 4b). For PVA, the initial mass loss due to moisture evaporation took place within the temperature range of 60–190 °C. The major loss (61.96%) occurred in between 239 °C to 374 °C which can be ascribed to the elimination of hydroxyl groups in the form of water and formation of polyene macromolecules [45]. The T_m of PVA was noticed at 305.06 °C. In the third step (410–476 °C), decomposition of macromolecules led to the mass loss of 10.98%. The batch HF0 exhibited degradation in two stages (I: 40–174 °C; II: 223–350 °C) with total weight loss of 62.3% at 485 °C whereas HF3 showed three stages of degradation (I: 30–176 °C; II: 234–343 °C; III: 418–480 °C) to due to presence of PVA. The total weight loss for HF3 at 485 °C was found to be 67.24% which was slightly greater than HF0. Also, T_m of HF3 was found to be reduced when compared with HF0. This clearly reveals that the crosslinking density of HF3 was less than HF0.

The values of the glass transition temperature (T_g) obtained from the DSC curves of HF0 and HF3 helped to reconfirm our above statement (see Fig. 4c). The T_g of HF3 was lower as compared to HF0 indicating decrease in its crosslinking density.

Thus, the results of carboxyl content and instrumental characterization were in complete agreement with each other.

3.6. Tensile strength of hydrogel films

The tensile strength of the hydrogel films was found to be increased with increase in the concentration of PVA up to 0.2% followed by decrease (see Table 2). The reason behind low value of tensile strength of CMC hydrogel films may be the lesser extent of crosslinking. The addition of PVA not only increases the extent of crosslinking but also enhances the hydrogen bonding interaction in between unreacted OH groups of PVA and, OH and carbonyl groups of other components (i.e.

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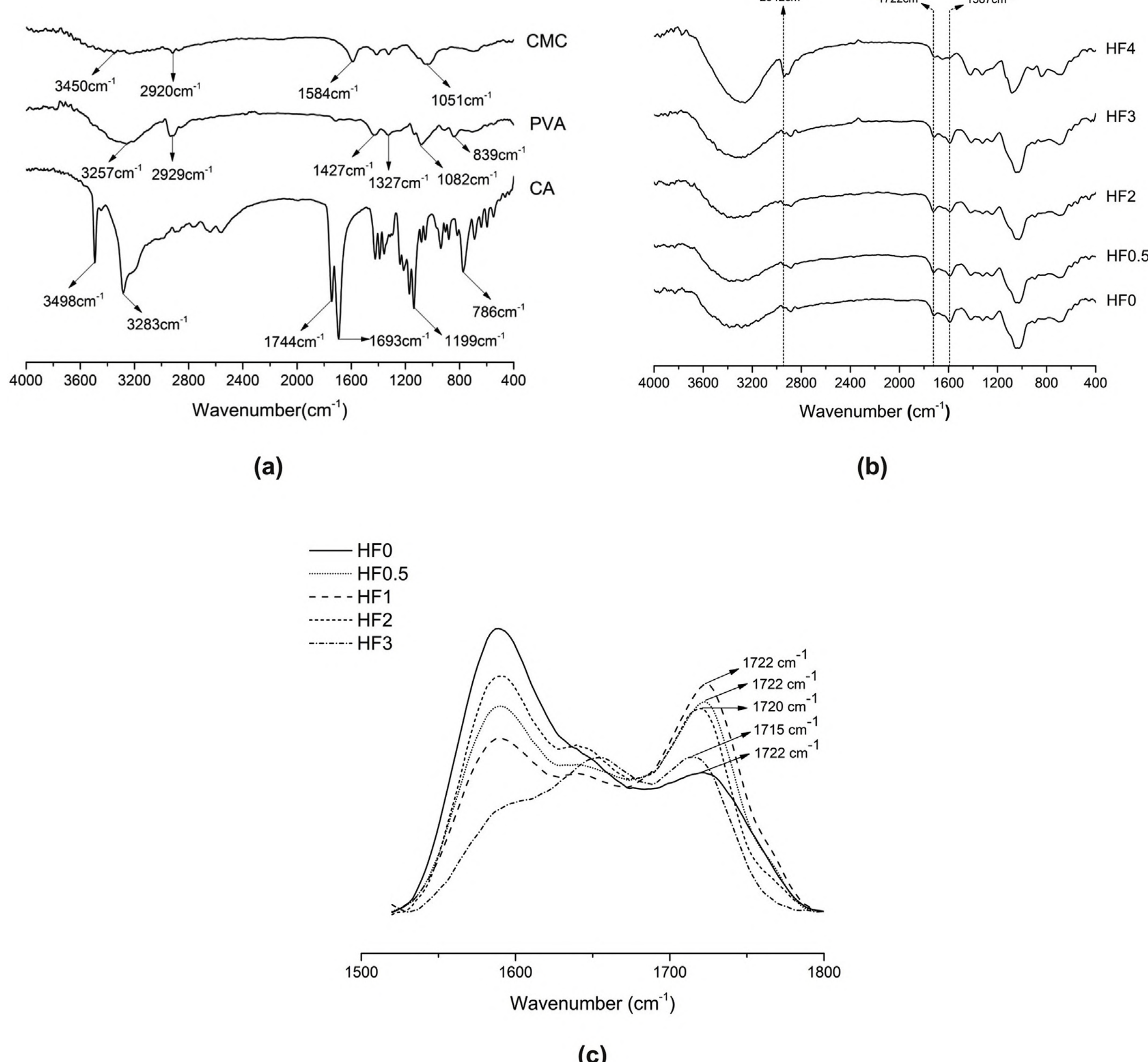

Fig. 3. ATR-FTIR spectra of CMC, PVA and CA (a), hydrogel films (b) and overlain spectra (baseline corrected) of hydrogel films in the region 1500 cm^{-1} to 1800 cm^{-1} (c).

CMC and CA crosslinks) within the CMC-PVA hydrogel films [46]. This can improve the mechanical property of the hydrogel films. The batch HF1 exhibited maximum tensile strength (3.54 ± 0.12 kg/mm^2) than the other hydrogel films. As the concentration of PVA was increased above 0.2%, the tensile strength of the CMC-PVA hydrogel films was found to be reduced slightly which can be attributed to the decrease in the crosslinking density of the hydrogel films. As compared to the pure CMC hydrogel film, CMC-PVA hydrogel films exhibited better mechanical strength.

3.7. Swelling study

Fig. 5 illustrates the swelling behavior of the hydrogel films. All the films showed initial increase in the swelling ratio till equilibrium followed by erosion. The pure CMC hydrogel films (HF0) showed minimum equilibrium swelling ratio (ESR) as compared to the CMC-PVA hydrogel films (HF0.5-HF3) (see Table 2). It was noted that despite of greater extent of crosslinking than HF0, HF0.5 and HF1 showed good swelling. This can be related to the increase in the total polymer concentration where PVA chains will be arranged in between the CMC chains. Secondly, on addition of PVA, the number of free OH groups in the hydrogel film will increase which can enhance the hydrophilicity of the hydrogel films [47]. An increase in the number of unreacted hydroxyl groups can be confirmed from an increase in the intensity of O-H stretching vibrations from HF0.5 to HF3 (see Fig. 3b). The other reason behind increase in the swellability of HF0.5 and HF1 than HF0 may be introduction of highly hydrophilic carboxylic groups due to increase in the number of crosslinks. These free carboxylic groups play an important role in the formation of a polyelectrolyte network within the hydrogel films and improve the water sorption [19]. The time taken by HF1 to reach the equilibrium (5 h) was found to be more than HF0 (4 h) which can be due to greater crosslinking density in HF1. A further increase in the concentration of PVA increased the swellability of the hydrogel films (HF2 and HF3) and also reduced the time required to achieve the equilibrium. This may be due to increase in the hydrophilicity and decrease in the crosslinking density of HF2 and HF3. HF3 showed maximum ESR than the other films (see Table 2).

3.8. GTM loading and release

The GTM loading increased with increase in the swellability of the hydrogel films and hence, the amount of PVA (see Table 2). The greater

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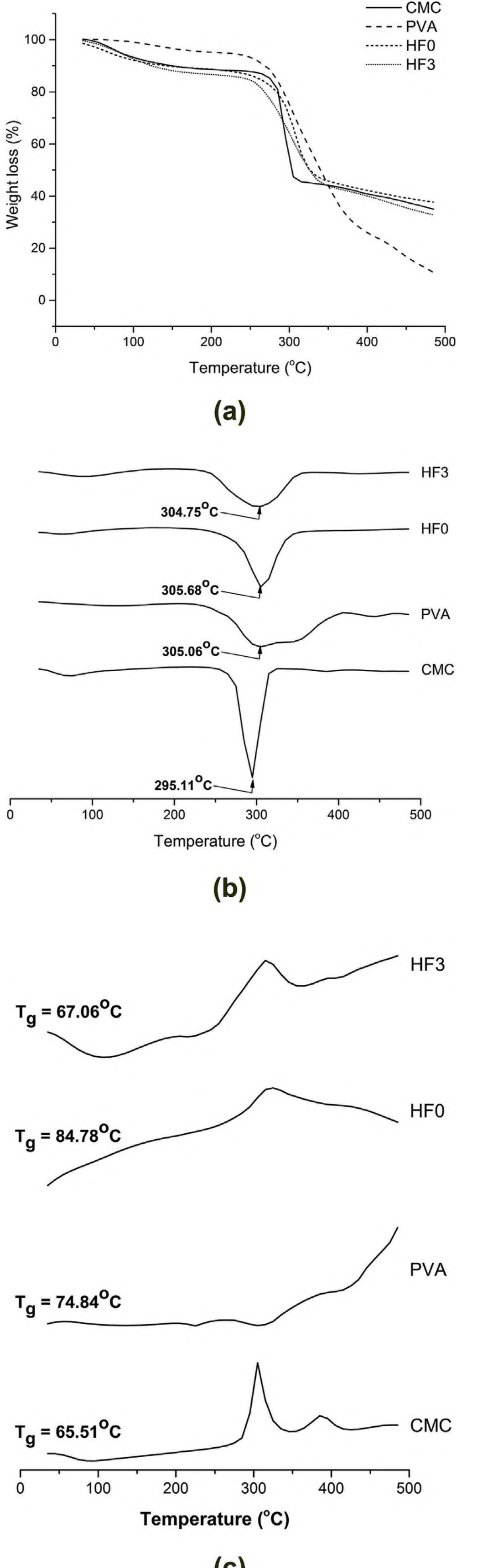

Fig. 4. TGA (a), DTG (b) and DSC (c) thermograms of CMC, PVA and hydrogel films (HF0 and HF3).

the swellability of the hydrogel films more will be their tendency to absorb the drug solution. As a result, large number of drug molecules will get entrapped in the polymeric network of hydrogels. The CMC-PVA hydrogel films showed significant improvement ($p < 0.05$) in the GTM loading than the CMC hydrogel film, with HF3 exhibiting maximum drug loading.

Besides swellability, hydrogen bonding interaction in between amine groups of GTM and hydrogel components (hydroxyl and carbonyl groups), and electrostatic interaction in between protonated amine groups of GTM and deprotonated carboxyl groups of hydrogel films may also contribute in enhancing the loading of GTM. In order to confirm the hydrogen bonding interactions, ATR-FTIR analysis of pure GTM and GTM loaded HF3 was performed (see Fig. 6). The spectrum of GTM showed absorption bands in the range of 2900–3500 cm^{-1} corresponding to N-H stretching vibrations of amino groups. Multiple peaks were found to be fused in the range of 2300–2900 cm^{-1} which represented C-H stretching and absorption due to the other components of GTM [48]. The peaks in the range of 1400–1700 indicated N-H bending, C-H stretching and C-N stretching vibrations. The strong band in the range of 900–1250 was related to C-N and C-O stretching vibrations. In spectrum of GTM loaded HF3, the N-H stretching bands of GTM nearly disappeared. Also, the broad peak of blank HF3 representing O-H stretching shifted from 3284 cm^{-1} to 3217 cm^{-1}, with decrease in its intensity. Such changes suggest the existence of hydrogen bonding interactions in between NH_2 groups of GTM and OH groups of HF3. As the number of OH groups increase from HF0 to HF3, the hydrogen bonding interactions in between GTM and hydrogel components will increase, thus enhancing the loading of GTM in the hydrogel films. The hydrogen bond formation is also possible in between amine groups of GTM and carbonyl groups of hydrogel films. The C-O stretching peak of HF3 at 1082 cm^{-1} shifted to lower wavenumber due to peak of GTM at 1037 cm^{-1}. The other peaks of GTM overlapped with the peaks of HF3 and therefore could not be distinguished.

Furthermore, electrostatic interaction in between GTM and free COOH groups of hydrogel films was possible because the drug loading was carried out in the aqueous drug solution of pH 5.8. At this pH, the amine groups of GTM undergo protonation (pKa = 8.6) whereas free carboxylic acid groups of hydrogel films are deprotonized. Consequently, electrostatic attraction will occur in between NH_3^+ groups of GTM and COO^- groups of hydrogel films, thus assisting in the drug loading improvement. As CMC-PVA hydrogel films exhibited greater carboxyl content than the pure CMC hydrogels, such electrostatic interaction will be more favored in their case.

An increase in the GTM loading in the CMC-PVA hydrogel films inspite of decrease in their carboxyl content indicates that the drug loading was mainly dependent upon swellability and hydrogen bonding.

Fig. 7 demonstrates the drug release profile of the GTM loaded hydrogel films. HF0 showed a noticeable burst release of GTM (26.96 ± 1.48%) within first hour, followed by rapid release. During drying of the drug loaded hydrogel films, most of the drug molecules diffuse along with the solvent towards the surface of the hydrogel films. As a result, a large number of drug molecules concentrate near the surface of the hydrogel films resulting into burst release. An increase in the concentration of PVA reduced the burst release due to an increase in the interaction between GTM and PVA (hydrogen and electrostatic bonding) within the hydrogel films. This will lead to maximum entrapment of GTM within the core of hydrogel matrix and concentration of the GTM molecules near the hydrogel surface will be reduced, thus reducing the burst release in case of CMC-PVA hydrogel films.

In case of HF0, nearly complete GTM release (98.90 ± 1.74%) occurred in 12 h. The CMC-PVA hydrogel films were capable of controlling the release of GTM above 24 h. The retardation of drug release was observed as we move from HF1 to HF3 which can again be attributed to the intermolecular interactions which can restrict the rapid transit of the GTM molecules from the hydrogel matrix into the release

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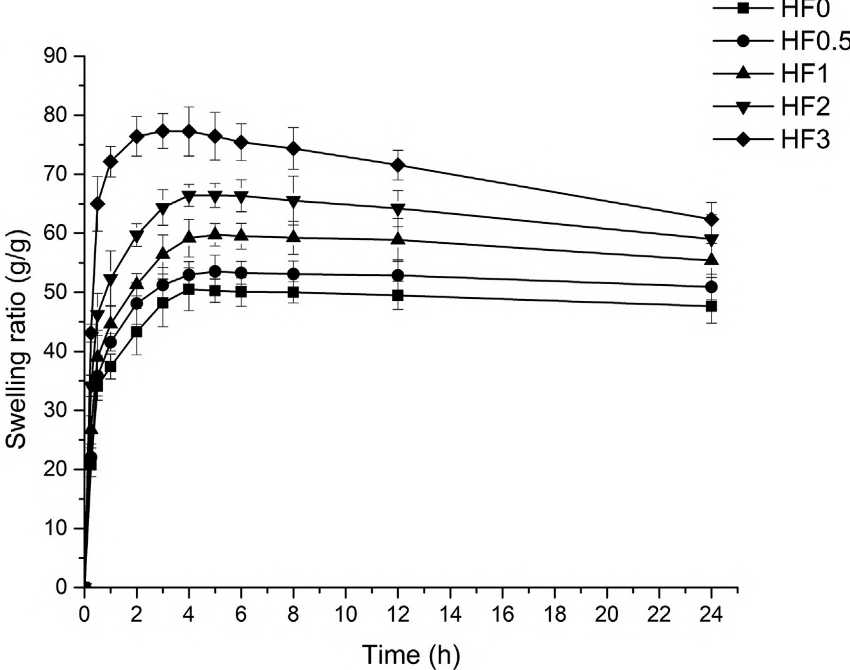

Fig. 5. Swelling profile of the hydrogel films (HF0-HF3).

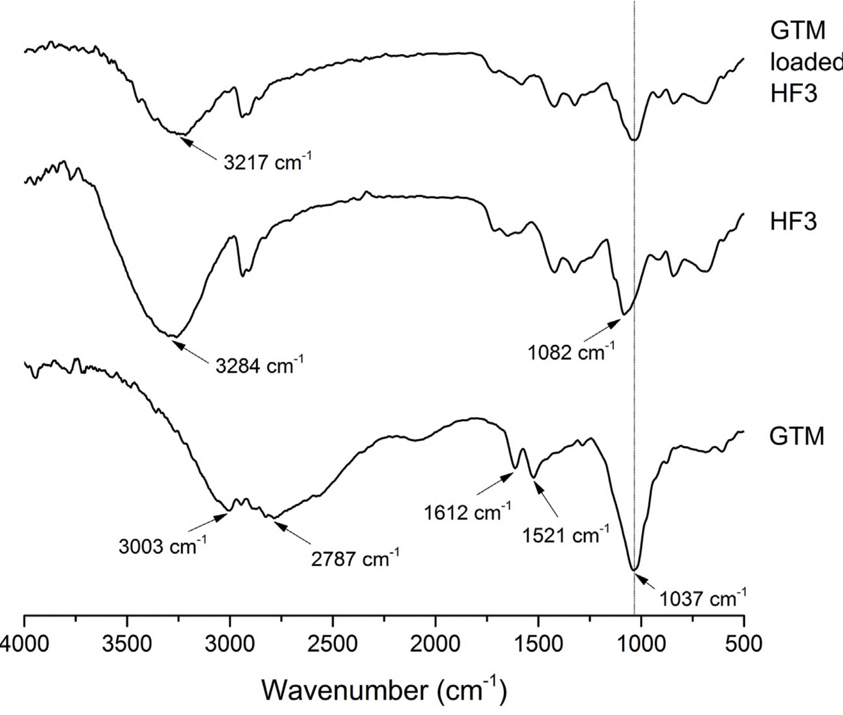

Fig. 6. ATR-FTIR spectra of GTM, HF3 and GTM loaded HF3.

medium. HF3 showed maximum drug release retardation (80.29 ± 2.22% at the end of 24 h) as compared to HF0.5 (98.41 ± 2.14% at the end of 24 h), HF1 (94.03 ± 2.86% at the end of 24 h) and HF2 (86.54 ± 1.94% at the end of 24 h). As HF3, showed maximum drug loading and had greater tendency to control the release of GTM than the other batches, it can be considered as optimized hydrogel film.

3.9. Hemocompatibility

The results of hemolysis assay are shown in Table 2. It was observed that the hemolysis due to pure CMC and CMC-PVA hydrogel films was less than 5% indicating hemocompatible nature of the hydrogel films [7]. From the values of percent hemolysis, it was difficult to determine the effect of PVA on the extent of hemolysis.

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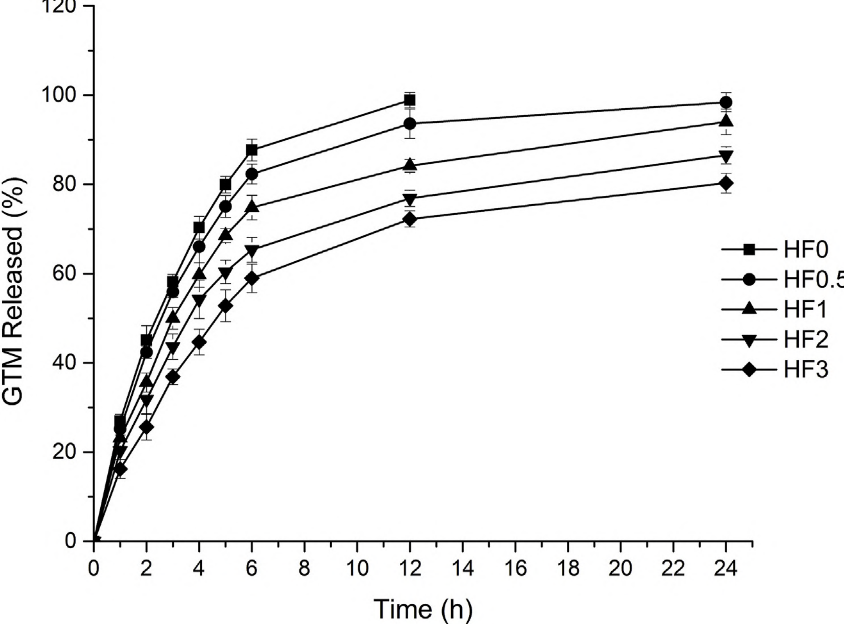

Fig. 7. GTM release (%) from the pure CMC and CMC-PVA hydrogel films.

4. Conclusion

The CA crosslinked CMC-PVA hydrogel films were formed by esterification-crosslinking mechanism. As the concentration of PVA in the feed was increased, the carboxyl content increased initially indicating increase in the crosslinking density, followed by decrease due to dilution effect. The presence of ester crosslinks and extent of crosslinking was confirmed from ATR-FTIR, solid state ^{13}C NMR and thermal analysis. The CMC-PVA hydrogel films showed good mechanical strength than the pure CMC hydrogel films. An increase in the amount of PVA in the hydrogel films improved the swellability of the hydrogel films and also enhanced the loading of GTM. CMC-PVA hydrogel films were capable of extending the release of GTM above 24 h. The prepared films were hemocompatible. Thus, CA crosslinked CMC-PVA hydrogel films exhibited potential to be used as an efficient and cheap biomaterial for delivery of water soluble basic drugs; however this can be confirmed only after conduction of few more tests such as MTT assay and in *vivo* studies.

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Conflicts of interest

Authors declare no conflict of interest.

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IMPORTANCE OF FORCED DEGRADATION STUDY IN PHARMACEUTICAL INDUSTRY— A REVIEW

Sandesh R. Chavan[1]*, Avinash M. Bhagwat[1], Mahesh Rao[2] and Atul P. Choudhari[3]

[1]Yashoda Shikshan Prasarak Mandal's, Yashoda Technical Campus, Faculty of Pharmacy (B. Pharm), Wadhe, Satara- 415011, Maharashtra, India.

[2]Assistant Manager, Micro Lab Ltd., Bangalore – 560 001, Karnataka, India.

[3]Lecturer (Selection Grade), Smt. S. S. Patil Institute of Pharmacy, Chopada, Jalgaon - 425 107, Maharashtra, India.

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***Corresponding Author**
Sandesh R. Chavan
Yashoda Shikshan Prasarak Mandal's, Yashoda Technical Campus, Faculty of Pharmacy (B. Pharm), Wadhe, Satara- 415011, Maharashtra, India.

ABSTRACT

Forced degradation is the process or state in which new drug substance and drug products are with the conditions which are more severe than accelerated stability conditions. The stability of drug substance is a critical parameter which may affect purity, potency and safety. Changes in drug stability can risk patient safety by formation of a toxic degradation product or deliver a lower dose than expected. Therefore it is essential to know the purity profile and behavior of a drug substance under various environmental conditions. The real and accelerated stability of the dosage forms are generally studied in pharmaceutical industry but force degradation study has its importance before starting a new formulation. It is required to demonstrate specificity of stability indicating methods and also provides an insight into degradation pathways of degradation products of the drug substance and helps in elucidation of the structure of the degradation products. Forced degradation studies show the chemical behavior of the molecule which in turn helps in the development of formulation and selection of packaging material. ICH guidelines demonstrate certain degradation conditions like light, oxidation, dry heat, acidic, basic, hydrolysis etc. ICH Q1A, QIB and Q2B exemplify the forced degradation studies. the present review discusses the current process and scenario in performance of forced degradation studies, its method and its importance in new formulation development.

KEYWORD: Degradation condition, Degradation product, Forced degradation, Stability.

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INDRODUCTION

A forced degradation study also known as stress testing, stress studies, stress decomposition studies, forced decomposition studies, etc. is the intentional degradation of the API or Drug Product to an appropriate extent by means of various stress conditions such as pH, temperature, light, oxidizing agents, mechanical stress etc. According to FDA guideline, a Force Degradation Study is defined as a validated analytical procedure that accurate and precisely measure active ingredients (drug substance or drug product) free from process impurities, excipients and degradation products. It is a quantitative test method that can detect possible degradants and impurities of drug substance (API) and drug products, normally using HPLC.[18] The main objective of stability indicating method is to monitor results during stability studies in order to guarantee safety, efficacy and quality. Before performing studies, a stability method is necessary so that any possible degradants generated during storage conditions (such as 30°C/60% RH and 40°C/75% RH) can be separated, detected and quantified. Forced degradation studies are used to identify reactions which may occur to degrade a processed product. It is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule. The stability studies include long term studies (12months) and accelerated stability studies (6months). As compared to stability studies, forced degradation studies help in generating degradants in much shorter span of time, mostly a few weeks.[15] The samples generated from forced degradation can be used to develop the stability indicating method which can be applied latter for the analysis of samples generated from accelerated and long term stability studies. As stated by United States Food and Drug Administration guidelines, a Stability-Indicating method] is defined as a validated analytical procedure that accurately and precisely measures active ingredients free from potential interferences like degradation products, process impurities, excipients, or other potential impurities, and the FDA recommends that all assay procedures for stability studies be stability indicating. The definition in the draft guideline of 1998 read as Validated Quantitative analytical methods that can detect the changes with time in the chemical, physical, or microbiological properties of the drug substance and drug product, and that are specific so that the contents of active ingredient, degradation products, and other components of interest can be accurately measured without interference. Stability-Indicating Method are validated quantitative test methods that can detect changes with time in the chemical, physical, or microbiological properties of drug substances or drug products. They are specific so that the quantity of the active ingredient,

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degradation products and other components of interest may be accurately measured without interference in the material being tested. A degradation product is a molecule resulting from a change in the active ingredient brought about over time as a result of processing or storage. The regulation requires a formal written stability testing program whose results are used to establish storage conditions and expiration dates of drug products and further mandates the use of reliable, meaningful, and specific test methods. A drug application is expected to contain a full description of the drug substance or drug product including its physical and chemical characteristics and stability as well as such specifications and analytical methods as are necessary to assure the identity, strength, quality, purity and bioavailability of the drug product, and stability data with proposed expiration dating. If such documentation is generated to support a regulatory submission such as an Investigational New Drug Application (IND), Drug Master File (DMF) or generated to satisfy cGMP requirements for a non-application drug substance or drug product. These data are used to establish, confirm or extend retest intervals (usually) or expiration dating periods (if unstable) for drug substances and expiration dating periods for drug products.[10]

FORCED DEGRADATION STUDIES

The ICH guideline Q1A on Stability Testing of New Drug Substances and Products gives indications for the testing of parameters which are may be susceptible to change during long storage and are likely to affect quality, safety and efficacy must be done by validated stability indicating testing methods. It is mentioned that forced degradation studies or stress testing at temperatures in 10 °C increments above the accelerated temperatures, extremes pH and under oxidative and photolytic conditions have to be carried out on the drug substance so to set up the stability characteristics and degradation pathways to back up the suitability of the proposed analytical procedures.

Objective of forced degradation studies

Forced degradation studies are carried out to achieve the following purposes:[14, 12, and15]

1. To establish degradation pathways of drug substances and drug products.
2. To differentiate degradation products that is related to drug products from those that are generated from non-drug product in a formulation.
3. To elucidate the structure of degradation products.
4. To determine the intrinsic stability of a drug substance in formulation.

5. To reveal the degradation mechanisms such as hydrolysis, oxidation, thermolysis or photolysis of the drug substance and drug product.
6. To establish stability indicating nature of a developed method.
7. To understand the chemical properties of drug molecules.
8. To generate more stable formulations.
9. To produce a degradation profile similar to that of what would be observed in a formal stability study under ICH conditions.
10. To establish degradation pathways and intrinsic stability of the drug molecule.
11. To validate stability-indicating analytical procedures.
12. To identify impurities related to drug substances or excipients.
13. To distinguish degradation products in formulations that is related to drug substances from those that are related to non-drug substances (e.g., excipients).
14. To solve stability-related problems (e.g., mass balance).
15. To generate a degradation profile that mimics what would be observed in a formal stability study under ICH conditions.
16. To facilitate improvements in the manufacturing process and formulations in parallel with accelerated pharmaceutical studies".
17. To choose the correct storage conditions, appropriate packaging and better understanding of the potential liabilities of the drug molecule chemistry".[16]
18. To facilitate improvements in the manufacturing process and formulations in parallel with accelerated pharmaceutical studies.[16]

Regulatory overview

ICH guidelines - regulatory overview

The ICH (The International Committee for Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use) has achieved harmonization in many areas of quality e.g. in conducting of stability studies or in providing definition of relevant thresholds for impurity testing. ICH has published several guidelines which have been discussed, agreed upon and adopted by the regulatory authorities of the ICH regions United States, Europe and Japan. When it comes to the topic "forced degradation" the most ICH guidelines emphasize the importance of conducting forced degradation studies, but provide only very general and limited information on the experimental stress conditions and only general guidance on how to conduct forced degradations studies. For example, the guidelines do not provide specific information and recommendations on the stress conditions e.g. pH,

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temperature ranges, specific oxidizing agents, or conditions to use. Furthermore, the guidelines mostly refer to new drug substances and drug products and do not refer to drug substances and clinical development.

Following ICH guidelines are in place and applicable when searching for guidance with regard to conducting forced degradation studies.
ICH Q1A – Stability Testing of New Drug Substances and Products.[16, 4]
ICH Q1B – Photo stability Testing of New Drug Substances and Products.[2]
ICH Q2B – Validation of Analytical Procedures: Methodology.[3]
ICH Q3A – Impurities in New Drug Substances.[16]
ICH Q3B – Impurities in New Products.[16]
M4Q (R1) – The common Technical Document (CTD).[9]

ICH Q1A – Stability Testing of New Drug Substances and Products.[16, 4]
In ICH Q1A (Stress Testing), there are recommended conditions for performing forced degradation studies on drug substances and drug products. The condition are to examine the effects of temperature (above that for accelerated testing, i.e. (>50°C), humidity (≥75% RH), oxidation and photolysis. Testing in solution should also be performed across a wide pH range either as a solution or suspension. These samples are then used to develop a stability-indicating method.

ICH Q1B – Photo stability testing of New Drug Substances and Products.[2]
ICH Q1B gives recommended approaches to assessing the photo stability of drug substances and drug products. Forced degradation conditions are specified in Section II (drug substance) and Section III (drug product). Exposure levels for forced degradation studies are not defined, although they can be greater than that specified for confirmatory (stability) testing. The actual design of photo stability studies is left to the applicant; however, scientific justification is required where light exposure studies are terminated after a short time, e.g., where excessive degradation is observed. Photo stability testing can be performed on the solid or in solution/suspension. These samples are then used to develop a stability indicating method. Both guidance's, Q1A and Q1B, note that some of the degradation products formed during forced degradation studies may not actually be observed to form during stability studies, in which case they need not be examined further.

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ICH Q2B–Validation of Analytical Procedures.[3]

ICH Q2B gives guidance on how to validate analytical methodology and in section B (impurities not available) there is a recommendation to use samples from forced degradation studies to prove specificity. Specificity is a key factor in determining whether or not the analytical method is stability indicating. Co-elution of peaks or components being retained on the column will underestimate the amount of degradation products formed and could compromise quality and increase risk to the patient.[3]

Q3A (R2) requires identification of each impurity with respect to both chemistry and safety perspectives. The chemistry perspectives include classification and identification of impurities, report generation, listing of impurities in specification and a brief discussion of analytical procedures while the safety perspectives include specific guidance for qualifying those impurities that were not present or were present at substantially lower levels in batch of a new drug substance and used in safety and clinical studies.[13]

Different forced degradation conditions utilized for drug substances and finished dosage form is shown in the "Fig. 1"[20] and Conditions commonly applied for forced degradation is tabulated in Table 1".

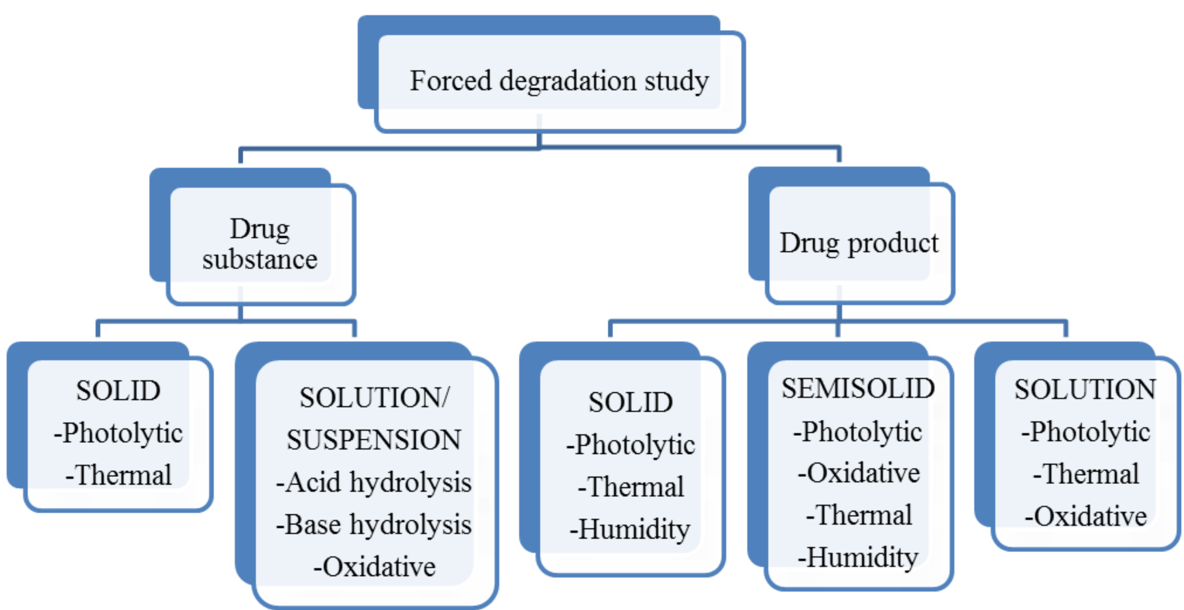

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Table 1: Condition usually used for forced degradation study.[10]

Degradation Type	Experimental condition	Storage condition	Sampling time
Hydrolysis	0.1N HCL	40°C, 60°C	1,3,5 days
	0.1N NaOH	40°C, 60°C	1,3,5 days
	pH:2,4,6,8	40°C, 60°C	1,3,5 days
Oxidative	3%H_2O_2	25°C, 60°C	1,3,5 days
	Peroxide control	25°C, 60°C	1,3,5 days
	Azobisisobutyronitrile(AIBN)	40°C, 60°C	1,3,5 days
Photolytic	Light,1 X ICH	NA	1,3,5 days
	Light,3 X ICH	NA	1,3,5 days
	Light control	NA	1,3,5 days
Thermal	Heat environment	60°C	1,3,5 days
	Heat environment	60°C/75%RH	1,3,5 days
	Heat environment	80°C	1,3,5 days
	Heat environment	80°C/75% RH	1,3,5 days
	Heat control	Room temperature	1,3,5 days

Appropriate Time to perform forced degradation

The forced degradation studies are not performed earlier it is very vital to conduct them during the phase III (FDA guidance states) to demonstrate the stability of a drug substance, potential degradation pathways and capability and suitability of proposed analytical procedures. Stress studies should be done in different pH solutions, in the presence of oxygen and light, and at elevated temperatures and humidity levels to determine the stability of the drug substance. The forced degradation studies conducted in a single batch. These studies are most useful if done initially in early development or phase I clinical trials which provides timely recommendations for improvement in the manufacturing process, ensure there is sufficient time for degradation product identification, proper selection of stability indicating analytical technique, degradation product identification and optimization of stress conditions which will help later in manufacturing process.

Limit of degradation

Usually degradation of drug substance between 5-20% is considered as reasonable and acceptable for validation of chromatographic assays. Some Pharmaceutical scientists have agreed that approximately 10% degradation is optimal for use in analytical validation. For small pharmaceutical molecules for which acceptable stability limits of 90% of label claim is common. In the event that the experimental conditions generate no or little degradation due to the experimental stability of the molecule, an evaluation should be made to verify if the drug

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substance has been exposed to energy in excess of the energy provided by accelerated storage (i.e., 40°C for six months).[10]

Selection of drug concentration

Which concentration of the drug should be used for the degradation study has not been specified in regulatory guidance. It is recommended that the studies should be initial concentration of 1 mg/ml. By using drug concentration of 1mg/ml it is generally possible to get even minor decomposition products in the range of detection. It is also suggested that some degradation studies should be done at a concentration which the drug is expected to be present in the final formulations.[15]

Degradation condition:

Typical stress tests include four main degradation mechanisms: heat, hydrolytic, oxidative, and photolytic degradation. Selecting suitable reagents such as the concentration of acid, base, or oxidizing agent and varying the conditions (e.g., temperature) and length of exposure can achieve the preferred level of degradation. Over stressing a sample may lead to the formation of secondary degradants that would not be seen in formal shelf-life stability studies and under-stressing may not serve the purpose of stress testing. Therefore, it is necessary to control the degradation to a desired level. A generic approach for stress testing has been proposed to achieve purposeful degradation that is predictive of long-term and accelerated storage conditions. The generally recommended degradation varies between 5-20% degradation. This range covers the generally permissible 10% degradation for small molecule pharmaceutical drug products, for which the stability limit is 90%-110% of the label claim.[6]

To know how much degradation is enough in stress testing forced degradation can be Classified into following types.[10]

1. Deceptive**:** Good degradation level (<15%) without any relevant degradants.
2. Predictive: Good degradation level (<15%) with one or more relevant degradants.
3. Useless**:** Between 15 to 100% degradation without any relevant degradants

1. Hydrolytic condition

Hydrolytic degradation (Acidic and basic hydrolysis): Hydrolytic degradation is one of the most frequent degradation chemical reactions over a wide range of pH. Hydrolysis is a chemical process that includes decomposition of a chemical compound by reaction with H2O. Hydrolytic degradation under acidic and basic condition involves catalysis of ionizable

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functional groups present in the molecule. Base or acid degradation testing involves forced degradation of a drug substance by exposure to acidic or basic conditions which generates primary degradants in desirable range. The selection of the type and concentrations of acid or base depends on the stability of the drug substance. HCl or H2SO4 (0.1 to 1 M) for acid hydrolysis and NaOH (or) KOH (0.1–1 M) for base hydrolysis are suggested as suitable reagents for hydrolysis. If the compounds for stress testing are poorly soluble in water, then co-solvents can be used to dissolve them in hydrochloric acid or Sodium hydroxide. Stress testing trial is normally started at room temperature and if there is no degradation, elevated temperature (50 – 70 °C) is applied. Stress testing should not exceed more than seven days. The degraded sample is then neutralized using suitable acid, base or buffer, to avoid further decomposition.[14, 8]

Procedure

For Acid hydrolytic study reflux with 0.1 N HCL at 60°C for 30 minutes. For Base stress reflux with 0.1N NaOH at 60°C for 30 min. For water stress Reflux with water at 60°C for 30 minutes.

2. Oxidative degradation

Hydrogen peroxide is widely used for oxidation of drug substances in forced degradation studies but other oxidizing agents such as metal ions, oxygen, and radical initiators (e.g., azobisisobutyroni-trile, AIBN) can also be used. Selection of an oxidizing agent, its concentration, and conditions depends on the drug substance. It is reported that subjecting the solutions to 0.1–3% hydrogen per-oxide at neutral pH and room temperature for seven days or up to a maximum 20% degradation could potentially generate relevant degradation products.[15] The oxidative degradation of drug substance involves an electron transfer mechanism to form reactive anions and cations. Amines, sulfides and phenols are susceptible to electron transfer oxidation to give N-oxides, hydroxylamine, sulfones and sulfoxide.[7] The functional group with labile hydrogen like benzylic carbon, allelic carbon, and tertiary carbon or α-positions with respect to heteroatom is susceptible to oxidation to form hydro peroxides hydroxide or ketone.[14]

Procedure

Treat with 3% H_2O_2 at less than 30°C for 30 min. The oxidative stress testing is initially carried out in 1% H_2O_2 at room temperature for 6 hr and it can be increased/ decreased to achieve sufficient degradation. Stress agent is changed to achieve degradation if necessary.

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3. Thermal degradation

Thermal degradation (e.g., dry heat and wet heat) should be carried out at more strenuous conditions than recommended ICH Q1A accelerated testing conditions. Samples of solid-state drug sub- stances and drug products should be exposed to dry and wet heat, while liquid drug products should be exposed to dry heat. Studies may be conducted at higher temperatures for a shorter period. Effect of temperature on thermal degradation of a substance is studied through the Arrhenius equation.[19]

$$A = ke^{-Ea/RT}$$

Where k is specific reaction rate, A is frequency factor, Ea is energy of activation, R is gas constant (1.987cal/degmole) and T is absolute temperature. Thermal degradation study is carried out at 40–80℃.[14, 15]

Procedure

Thermal degradation can be conducted based on physical properties of API i.e. Melting Point. If melting point of API is less than 150°C, stress at 105°C or 40°C less than melting point whichever is higher. If melting point of API is more than 150°C stress at the nearest melting point and at 105°C.

4. Photolytic degradation

Photo stability studies are performed to generate primary degradants of drug substance by exposure to ultra violet or fluorescent conditions. The rate of photo degradation depends upon the intensity of incident light and quantity of light absorbed by the drug molecule. Samples of drug substance and solid/liquid drug product should be exposed to a minimum of 200 W h/m2 light. The most commonly accepted wavelength of light is in the range of 300 - 800 nm to cause the photolytic degradation. Light stress conditions can induce photo oxidation by free radical mechanism. ICH guideline options requirements widely regarded. UV exposure - NLT 200 watts (sq meters). Visible exposure- NLT 1.2 Million lux-hrs. Functional groups like carbonyls, nitro aromatic, N-oxide, alkenes, aryl chlorides, weak C-H and O-H bonds, sulfides and polyenes are likely to introduce drug photosensitivity. Options per ICH Q1B: Any light source with output similar to D65/ID65 emission standard, such as (i) artificial daylight fluorescent lamp combining visible and UV outputs (ii) xenon lamps or (iii) metal-halide lamps. A cool white fluorescent lamp per ISO 10977 and a near UV fluorescent lamp

having a spectral distribution 320 - 400 nm with a maximum energy emission 350 - 370 nm. A significant portion should be in both bands 320 - 360 nm and 360 - 400 nm.[14]

Procedure

Expose the tablet powder/content of capsule to intense ultraviolet radiation (both at longer and shorter wavelengths) up to minimum of 7 days in UV cabinet.

Acceptance criteria

All requirements of the software are to be met while evaluating peak purity. The purity angle should be less than purity threshold.[5] Peak purity not less than 0.995.[21] If peak purity not observed within the limit this molecule is sensitive for specific condition.

Mass balance of all stressed samples shall be verified by calculating

Mass balance: (% assay of stressed sample +% impurities) X 100/ % assay of unstressed sample.

-mass balance is to be achieved at least up to 95% levels.[11]

-if the mass balance is less than the required criteria investigation to be done and justified.[11]

Analytical tools for separation and identification of degradant

A. Convectional technique[15]

1. Thin layer chromatography (TLC).
2. Solid phase extraction (SPE)
3. Accelerated solvent extraction (ASE)
4. Low-pressure Liquid Chromatography (Flash chromatography)
5. Supercritical fluid extraction (SFE) countercurrent chromatography (CCC)
6. Mass Spectrometry (MS).
7. NMR: NMR spectroscopy is an extremely powerful tool for the analysis of drug degradation products. In order to perform NMR-based structure elucidation of drug degradant products, it is common practice to isolate sufficient material (>1 mg) for NMR analysis.

8. HPLC

HPLC is routine technique for separation of degradants. The normal UV HPLC detectors these days allow for simultaneous measurement at multiple wavelengths, and some of them

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even give output of ratio plots at two wavelengths. This technique has also been promoted for peak purity testing during development of SIMs.

B. Hyphenated technique[15]

1. GC-MS.
2. LC-MS.
3. Capillary Electrophoresis- Mass Spectrometry (CE-MS).
4. Liquid chromatography-Fourier Transfer Infrared (LC-FTIR)
5. LC-NMR: The advantages of using NMR in combination with HPLC in comparison to HPLC-MS coupling are (1) both HPLC and NMR are conducted in solution and no transfer from one phase to another, as from the liquid to vapour phase in HPLC-MS; (2) NMR measurements are not limited by vaporization and hence by molecular weight; (3) in many cases the structure information by NMR spectra is more extensive, especially when the stereochemistry of the molecule is considered.

Evaluation of forced degradation

Peak purity

Peak purity is comparison of the reference standard to the API in the sample stressed by “forced degradation”. it is used as a support in stability indicating method development. The spectral uniqueness of a compound is used to establish peak purity when co eluting compounds are present. Limitations to peak purity arise when co eluting peaks are spectrally similar, or below the detection limit, or a peak has no chromophore, or when they are not resolved at all.

Mass Balance

Mass balance is calculated by adding the assay value and the amounts of impurities and degradants to evaluate the closeness to regulatory guidance for forced degradation, It is recommended to use appropriate conditions to achieve 5-20% degradation. Success to formulation depends on degradation study and it absolutely depends on skill of researcher, so force degradation study is very important tools for the new formulation development.

CONCLUSION

Forced degradation studies provide knowledge about possible degradation pathways and degradation products of the active ingredients and help elucidate the structure of the degradants. It is essential to help to develop and demonstrate specificity of stability-

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indicating methods. Forced degradation is important part of the formulation development process as it provides knowledge about the degradation, chemistry of drug substances and drug products. This knowledge is used to develop analytical method, formulation development, packaging development and the design of the official stability studies. They were also useful in the investigation of the chemical and physical stability of crystal forms, the stereo chemical stability of the drug substance alone and in the drug product and mass-balance issues, and for identifying drug related degradation products in formulations. Stress testing has played a critical role in the drug development process, there is no formal regulatory guidance for forced degradation, it is recommended to use appropriate conditions to achieve 5-20% degradation. Success to formulation depends on degradation study and it absolutely depends on skill of researcher, so force degradation study is very important tools for the new formulation development.

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STUDY OF FRUCTOSE-GLUCOSE RATIO IN DIFFERENT SAMPLES OF HONEY AVAILABLE IN SATARA REGION

Shivani R. Ghadge[1]*, Avinash M. Bhagwat[1], Satish S. Deshpande[2] and Sambhaji K. Budhavale[3]

[1]Yashoda Shikshan Prasarak Mandal's, Yashoda Technical Campus, Faculty of Pharmacy (B. Pharm), Wadhe, Satara- 415011, Maharashtra, India.

[2]Production Manager, Ayurvedeeya Arkshala Ltd; Satara – 415 002, Maharashtra, India.

[3]Associate Professor, Rajgadh Dnyanpeet's College of Pharmacy, Bhor, Dist- Pune 412 206, Maharashtra, India.

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***Corresponding Author**
Shivani R. Ghadge
Yashoda Shikshan Prasarak Mandal's, Yashoda Technical Campus, Faculty of Pharmacy (B. Pharm), Wadhe, Satara- 415011, Maharashtra, India.

ABSTRACT

The main objective of this project work was to determine fructose-glucose ratio in different honey samples. In this project, we tested 7 different samples of honey available in Satara region for its fructose-glucose ratio. The determination of fructose and glucose ratio ultimately gives the % by weight of glucose and fructose. As we know that sugar is unhealthy in excess to diabetes patients and hence comparison between regular sugars and honey must be done so that we can give a better alternative to regular sugar in a healthy way. Honey fructose and purified fructose plays different roles in our body in a very different way. By studying the percent reducing sugars in honey we are trying to conclude that how honey can be a good and healthy sweetener as that of other sweeteners which are causing hazardous effects on our body and health.

KEYWORDS: Honey, Fructose, Glucose, Fructose-Glucose ratio, Reducing sugars, and Honey Fructose.

INTRODUCTION

What Is Honey?

Honey is naturally occurring sweet fluid produced by the honeybees by enzymatic transformation of floral nectar ingested by them and deposited in the cells of hives or combs.

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The Indian species of honeybees belongs to the genus *Apis* of which the common ones are *A. florea* (Family-Apidae). In commerce, Honey may be collected from naturally occurring hives in grooves and forests, by pressing and squeezing in the traditional method or may be centrifugation of the combs containing honey in artificially maintained apiaries. Both should be filtered before storage or use.[1]

Synonyms

Puspasava, Pusparasa, Ksaudra, Madhvika, Madhu.[1]

Description

A thick, syrupy, translucent, yellow to yellowish brown fluid; tastes sweet with a pleasant odour and flavor. When poured on to a tray as a thin layer, no impurities like mould, dirt, beeswax, insect fragments, plant debris or any other objectionable foreign matter should be visible to the naked eye in daylight.[1]

Honey has been 'nature's sweetener' for centuries and is frequently marketed as 'superior' to sugar. Honey has been a traditional sweetener for thousands of years long before we discovered how to extract and refine sugar from sugar cane or sugar beet.

Today we consume over 40 times more sugar than honey yet it remains a favourite flavouring in foods like honey cakes, sauces, breakfast cereals, honey-coated nuts, yoghurts etc.

Sugar is 100 per cent sucrose, while honey is made up of around 75 per cent sugars, of which roughly half is glucose and half is fructose (these proportions may vary depending on the source of the nectar). The remaining 20 to 25 per cent is water with a trace of protein, a trace of fat and a trace of fibre, small amounts of plant acids, waxes, gums, pigments and volatile oils which have antioxidant and antibacterial properties. Honey also contains vitamin B and minerals but in very less quantity and not nutritionally significant.[4]

Purpose of the Study

The main purpose of this study to determine fructose-glucose ratio in honey of different brands available in satara region.

This will ultimately lead to the information about the honey, fructose-glucose ratio, advantages of honey over regular sugar and how to maintain a good and healthy diet.

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Research Questions

During this study, many questions came in our way to find out importance of this study like,

- Is honey healthier than sugar?
- What are the honey health benefits?
- What is fructose- glucose ratio?
- Why it is so important?
- What is the role of fructose and glucose in our body?
- How fructose-glucose affects our health?

Is Honey Healthier Than Sugar?

Honey once described as 'nectar of the gods' and is often considered a 'natural', healthier sweetener than sugar but nutritionally its true advantages are minor.

As we said before sugar is 100 per cent sucrose, honey is made up of around 75 per cent sugars, of which roughly half is glucose and half is fructose. The remaining 20 to 25 per cent is water with a trace of protein, a trace of fat and a trace of fibre, which explains why honey has fewer 'sugars' or kilojoules/calories than sugar when you compare them weight for weight.

Comparison of sugar vs. honey:-

100g white sugar :- 1700kJ/406Cals.

100g of honey :- 1400kJ/334Cals.

But in day-to-day life most of us we eat honey a teaspoon or a tablespoon not by its weight. And hence as the honey is denser than sugar 1 tablespoon of honey weighs 28g, whereas a tablespoon of sugar weighs only 16g. So, if you are taking one tablespoon of honey instead of sugar you are consuming more calories than regular sugar.[4]

What Are the Honey Health Benefits?[2]

- Useful in weight management
- Strengthens immune system
- Nourishes your skin and face
- Boosts your memory
- Home remedy for cough
- Natural home remedy for Dandruff

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- Used for healing wounds
- Acts as natural sleeping aid
- Eases sinus issues
- Help with gum diseases
- Natural energy drink
- Prevents and helps control Eczema and many more.

What Is Fructose-Glucose Ratio?

Usually sugar contains Fructose and glucose in linked chain system, which has approximately half fructose and half glucose.

Glucose is simple sugar which is main energy source for body cells.

Fructose is a type of simple sugar that makes up 50% of table sugar (sucrose).

However, fructose needs to be converted into glucose by the liver before it can be used by the body. And hence limited quantity of fructose should be consumed to avoid its harmful effects.

Fructose-Glucose ratio gives the values of fructose and glucose so that we can determine its quantity and could take diet in appropriate measured quantities or could limit the diet in a healthy way.

Why Fructose-Glucose Ratio Is Important?

Basically fructose-glucose ratio calculated from fructose and glucose values present in the sample.

Fructose and glucose both are the simple sugars which provide quick energy and can be easily utilized by body cells for energy production.

Both sugars are good for health only in a safe and limited quantity but become very harmful when consumed in higher quantity.

As we have seen that glucose directly get absorbed in blood and raises the blood sugar level very quickly whereas fructose for absorption need to be metabolized by liver for absorption.

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As the fructose is twice sweeter than glucose most of the sugary sweeteners and added sugars in soft drinks, desserts and in bakery sweet food products contains high fructose in it. Before the mass production of refined sugar, humans rarely consumed it in high amounts. While some sweet fruits and vegetables contain fructose, they provide relatively low amounts.

High glucose consumption is unhealthy and leads to the type 2 diabetes and heart diseases. While excessive fructose is undoubtedly unhealthy, its health effects are controversial.

And hence glucose and fructose levels must be within the safe range so the determination of fructose and glucose levels becomes very important when it came to the health.[3]

What Is The Role Of Fructose And Glucose In Our Body?

Where high fructose level leads to the various harmful diseases such as ……

Mal-absorption: - Some people do not absorb all the fructose they eat. This condition is known as fructose mal-absorption, which is characterized by excessive gas and digestive discomfort. All carbohydrates, even rapidly digestible sucrose, cause abdominal discomfort when consumed to excess. Some individuals may exceed their capacity for fructose absorption if large amounts are eaten. Under such circumstances, fructose may be associated with abdominal complaints, such as bloating and flatulence. In those with fructose mal-absorption, fructose acts as a fermentable carbohydrate and is categorized as a FODMAP.[3]

Appetite:-Fructose does not suppress appetite in the same way as other sugars, fructose does not cause rapid surges and dips in blood glucose levels, which is one factor thought to stimulate eating.[3]

And hence the determination of both fructose and glucose becomes very necessary.

Unlike glucose, fructose causes a low rise in blood sugar levels. Therefore, some health professionals recommend fructose as a “safe” sweetener for people with type 2 diabetes.

However, others are worried that excessive fructose intake may contribute to several metabolic disorders as follows.[3]

- Glucose and fructose are metabolized very differently by the body.
- While every cell in the body can use glucose, the liver is the only organ that can metabolize fructose in significant amounts.

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- When people eat a diet that is high in calories and high in fructose, the liver gets overloaded and starts turning the fructose into fat.
- Impair the composition of your blood lipids. Fructose may raise the levels of VLDL cholesterol, leading to fat accumulation around the organs and potentially heart disease.
- Increase blood levels of uric acid, leading to gout and high blood pressure.
- Cause deposition of fat in the liver, potentially leading to non-alcoholic fatty liver disease.
- Cause insulin resistance, which can lead to obesity and type II diabetes.
- Fructose doesn't suppress appetite as much as glucose does. Thus, it might promote overeating.
- Excess fructose consumption may cause leptin resistance, disturbing body fat regulation and contributing to obesity.

Note: - It's important to realize that all of this does not apply to whole fruit.

Fruits aren't just watery bags of fructose; they are real foods with a low-calorie density and lots of fiber. They're hard to overeat on and you would have to eat very large amounts to reach harmful levels of fructose. In general, fruit is a minor source of fructose in the diet compared to added sugars.

The harmful effects of fructose apply to a Western diet supplying excess calories and added sugars. It does not apply to the natural sugars found in fruits and vegetables.[3]

How Fructose And Glucose Affects Our Health?

Many soft drinks, desserts, beverages, high fructose corn syrup and bakery food products use high amounts of fructose in it.[20]

Some have suggested that a rise in the use of HFCS in the United States (US) over the past 30 years could explain the rise in obesity and type II diabetes, and that this is due to increased fructose consumption.[5,20]

A recent study claimed that High-Fructose Corn Syrup (HFCS) causes obesity while sucrose does not, this study was thoroughly unconvincing. Over 8 weeks, rats with 24-hour access to control chow, sucrose, or HFCS had no difference in body weight. Rats with 12-hour access to HFCS had increased body weight, and rats with 12-hour access to sucrose did not. They didn't restrict any of the control rats to 12-hour access.

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Their main finding was that over seven months, female rats with 24-hour access to HFCS had increased abdominal and uteral fat and increased blood levels of triglycerides, but rats with 12-hour access to sucrose or HFCS did not.

Speaking of honey, however, research suggests that the fructose in honey doesn't behave anything like the fructose in refined sweeteners. Isn't that a sweet surprise!

Researchers fed weanling rats for two weeks on diets that were 65% (by weight of dry matter) starch, honey, or purified glucose and fructose purchased from Sigma. They provided glucose and fructose at the same ratio at which they occur in honey.[5]

And the findings were surprising such as[5] ….

- Purified fructose increased triglyceride levels, as expected. Honey seemed to increase triglyceride levels, but the increase was not statistically significant.
- Purified fructose, but not honey fructose, decreased blood levels of vitamin E. This suggests that it promoted oxidative stress.
- Purified fructose, but not honey, seemed to promote inflammation. Nitric oxide is very important to blood vessel function in small amounts, but large increases are usually a sign that immune cells have been activated to create an inflammatory state. Honey fructose just doesn't seem to promote inflammation the way purified fructose does.
- After all this they took some heart tissue and mixed it with iron sulfate and vitamin C. The combination of high doses of iron and vitamin C can create oxidative stress. This test shows how susceptible the heart tissue would be to suffering damage in the face of oxidative stress. Once again, purified fructose poses harm while honey fructose does not.[5]

MATERIALS AND METHOD

Methodology for Study of Fructose-Glucose Ratio in Different Honey Samples

Determination of Total Reducing Sugars

Total Reducing Sugars[1]

Reagent

Soxhlet modification of Fehling's solution – Prepare by mixing equal volumes of solution A and solution B immediately before using.

Copper Sulphate solution (Solution A) – Dissolve 34.639 g of copper sulphate crystals in water, dilute to 500 ml and filter through glass wool or filter paper.

Standardization of copper sulphate solution – Using separate pipettes, pipette accurately 5 ml of Solution A and 5 ml of solution B into a conical flask of 250 ml capacity. Heat this mixture to boiling on an asbestos gauze and add standard inverted sugar solution from a burette, about one milliliter less than expected volume which will reduce the fehling solution completely 48 ml. Add one ml of methylene blue indicator while keeping the solution boiling. Complete the titration within 3 minutes, the end point being indicated by change of color from blue to red. From the volume of invert sugar solution used, calculate the strength(S) of the copper sulphate solution by multiplying the titre value by 0.001 (mg/ml of standard invert sugar solution). This would give the quantity of invert sugar required to reduce copper in 5ml copper sulphate solution.

Potassium sodium tartrate (Rochelle Salt) solution (solution B) - Dissolve 173g of potassium sodium tartrate and 50 g of sodium hydroxide in water, dilute to 500 ml. let the solution stand for a day and filter.

Hydrochloric acid: – specific gravity 1.18 at 20’C (approximately 12 N).

Standard invert sugar solution

1. Weigh accurately 0.95 g sucrose and dissolve it in 500 ml water.
2. Add 2 ml of concentrated hydrochloric acid. Boil gently for 30 minutes and keep aside for 24 hours.
3. Neutralize with sodium bicarbonate and make the final volume to 1000 ml; 50 ml of this solution contains 0.05 g invert sugar.

Methylene blue indicator: – 0.02% in water.

Procedure[1]

1. Place accurately weighed about 1 gram (W) of prepared sample of honey into a 250 ml volumetric flask and dilute with about 150 ml of water.
2. Mix thoroughly the contents of the flask and make up volume to 250 ml with water.
3. Using separate pipettes, take accurately 5 ml of solution A and solution B, in a porcelain dish.

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4. Add about 12 ml of honey solution from a burette and heat to boiling over an asbestos gauze.
5. Add 1 ml methylene blue indicator and while keeping the solution boiling complete the titration, within 3 minutes.
6. The end point being indicated by change of color from blue to red.
7. Note the volume (H) in ml of honey solution required for the titration.[1]

Calculations

A. Total reducing sugar % by mass = $\frac{250\times100\times S}{H\times M}$

Where,

S = strength of copper sulphate solution,

H = volume in ml of honey solution required for titration, and

M= mass in gm of honey.

Fructose-Glucose Ratio[1]

Reagents

Iodine solution: – 0.05 N.

Sodium hydroxide solution: – 0.1 N.

Sulphuric acid: – concentrated.

Standard sodium thiosulphate solution: – 0.05 N.

Procedure[1]

1. Pipette 50 ml of honey solution in a 250ml stoppered flask.
2. Add 40 ml of iodine solution and 25ml of sulphuric acid.
3. Stopper the flask and keep in dark for 20 minutes.
4. Acidify with 5ml of sulphuric acid and titrate quickly the excess of iodine against standard sodium thiosulphate solution.
5. Conduct a blank using 50ml of water instead of honey solution.[1]

Calculations

A. Approximate glucose,

% by mass (w) = $\frac{(B-S)\times0.004502\times100}{a}$

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Where,

B = volume of sodium thiosulphate solution required for the blank,

S = volume of sodium thiosulphate solution required for the sample, and

a = mass of honey taken for test.

B. Approximate fructose, % by mass (x) =

$$\frac{\text{Approximately total reducing sugars, \% - w}}{0.925}$$

C. True glucose, % by mass(y) = w – 0.012 x

D. True fructose % By mass (z) =

$$\frac{\text{Approximate reducing sugars, \% - y}}{0.925}$$

E. True reducing sugars, % by mass = y + z

F. Fructose – Glucose Ratio =

$$\frac{\text{True Fructose, \% by mass (z)}}{\text{True Glucose, \% by mass (y)}}$$

OBSERVATIONS AND READINGS

Readings for Total Reducing Sugars

Sr. No.	Sample code	Readings (ml)			Mean (ml)
1	A	15	14.7	15	15
2	B	17.7	17.7	17.8	17.7
3	C	16.2	16	16.2	16.2
4	D	16.6	16.8	16.7	16.7
5	E	17	17	17.1	17.0
6	F	16.7	17.1	16.9	16.9
7	G	16.7	16.7	16.7	16.7

From the formula given for determination of total reducing sugars calculated are as follows:

- Strength of Copper Sulphate was found to be = 0.055 mg/ml.
- Calculation Results for Total Reducing Sugar in different samples is as follows: -

1. Total Reducing Sugar % by Mass of sample A = 91.66
2. Total Reducing Sugar % by Mass of sample B = 77.68
3. Total Reducing Sugar % by Mass of sample C = 84.87
4. Total Reducing Sugar % by Mass of sample D = 82.33
5. Total Reducing Sugar % by Mass of sample E = 80.88

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6. Total Reducing Sugar % by Mass of sample F = 81.36
7. Total Reducing Sugar % by Mass of sample G = 82.33

Readings for Fructose-Glucose Ratio

Sr. No.	Sample code	Readings (ml)			Mean (ml)
1	A	93.9	94.5	94.2	94.2
2	B	112.3	112	112	112
3	C	97	96.5	97	97
4	D	96.5	97.1	96.8	96.8
5	E	100	100.5	100.5	100.5
6	F	97	97	97	97
7	G	98.2	98.2	98.3	98.2

- Sodium Thiosulphate require for Blank was (B) = 120ml.

By above given formulas % Glucose, % Fructose, True Glucose, True Fructose and Fructose-Glucose ration is calculated and mentioned in results.

RESULTS AND DISCUSSION

Here in this project I perform the experimental work by process given above and it is the standard procedure for determination of fructose- glucose ratio in honey referred from the Aayurvedic Pharmacopeia.

Standard Values

Reducing sugars: – not less than 65% by wt.

Fructose-Glucose ratio: - not less than 1% by wt.

I took different 7 samples of honey from different store in Satara region.

By performing the given standard procedure, the obtained results and readings after calculating are enlisted in table given below….

Table 1: Fructose-Glucose ratio and other sugars in honey.

Parameters	A	B	C	D	E	F	G
Total reducing sugar	91.66	77.68	84.87	82.33	80.88	81.36	82.33
Glucose% (y)	45.87	13.58	40.85	41.25	34.52	40.90	38.69
Fructose % (z)	49.49	69.29	47.58	44.40	50.11	43.71	47.16
y + z	95.37	82.87	88.43	85.66	84.63	84.64	85.86
Fructose-Glucose ratio	1.07	5.10	1.16	1.07	1.45	1.06	1.21

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In this project the final results and ranges of % of fructose, glucose and reducing sugars in different honey samples found to be within the standard values and limits.

There were no adulterations found in honey samples.

As honey contains sugar level in limited quantity and hence won't show more harmful effects as that of the regular sugar.

Honey contains sugars along with various plant metabolites, antioxidants and other various enzymes hence it provides sweetness in a healthy way.

Variables

- Fructose-glucose ratio also depends on types of honey.
- Honey can be obtained by honeycomb but bee's collects honey from various flowers and hence exact and only specific flower nectar can't be obtained in honey.
- Honey of marketed products can be adulterated and hence the sugar levels may vary.
- Other formulation processes of honey in industry like pasteurization may affect the heat sensitive compounds and hence will affect in proportions.

CONCLUSION

- Fructose – Glucose ratio in different samples of honey available in Satara region is within normal range and limits.
- Total reducing sugars in different samples of honey are within standard limits.
- As honey contains sugars in limited quantity and also contains other plant metabolites, enzymes etc. it gives additional healthy effects along with the sweetness.[4]
- Honey fructose does not give same side effects as that of the purified fructose and hence become a better choice of sweetener than the regular sugars.[5]

Future of project

As we have concluded that honey can be a better option for sweetness than that of the regular sugars and various sweeteners available in market. But exactly how honey is beneficial? Why honey fructose does not gives same side effects as that of the purified fructose is the big question?

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Formulation and Evaluation of Herbal Scrub Gel

Pawar Dhanashri N., Pawar Arti P., Dalvi Yogita V.*
Assistant Professor, YSPM YTC Faculty of Pharmacy, Satara, (MH) India-415004

*Corresponding Author E-mail: yogitav.dalvi@gmail.com

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Abstract

Most of the marketed cosmetics when applied on the skin cause dryness of skin after its long term use which results less life of skin problems of acne and redness. Solution for this problem is use of scrub gel once or twice in week which consist all herbal ingredients which increases cleansing, softening, moisturizing, fairness of skin. In the present work we have formulated the herbal facial scrub by using a different herbal powders and it was evaluated by using the parameters like smoothness, appearance, spreadibility, irritation etc.

Top

Keywords

Scrub gel, softening, and cleansing, moisturizing, fairness.

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Formulation and Evaluation of Polyherbal Soap

Pawar Arti P.[1], Pawar Dhanashri N.[1], Dalvi Yogita V.[2,*]
[1]YSPM YTC, Faculty of Pharmacy, Satara, (MH) India-415 004

[2]Assistant Professor,YSPM YTC, Faculty of Pharmacy, Satara, (MH) India-415 004

*Corresponding Author E-mail: yogitav.dalvi@gmail.com

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Abstract

Polyherbal soap was prepared by using sandal wood and Orange peel extract and evaluated by using various evaluation parameters such as organoleptic characteristics, pH, foam height and retention, skin irritation and high temperature stability. Prepared Polyherbal soap having good appearance better cleansing and foaming effect and doesn't have any side effects.

Top

Keywords

Polyherbal soap, cleansing, foaming.

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Delaying Effect of Polyherbal Formulation on Cataract in STZ-NIC-induced Diabetic Wistar Rats

K. K. MALI*, SUCHETA S. LIGADE[1] AND R. J. DIAS[2]

Department of Pharmaceutics, YSPM'S Yashoda Technical Campus, Faculty of Pharmacy, Wadhephata, Satara-415 011, [1]Department of Pharmacology, Sant Gajanan Maharaj College of Pharmacy, Gadhinglaj, Kolhapur-416 503, India, [2]Department of Pharmacy, Government Polytechnic, National Highway No 6, Jalgaon-425 002, India

The aim of the study was to evaluate the effectiveness of polyherbal formulation on cataract development in streptozotocin-nicotinamide-induced diabetic rats. Diabetes was induced by streptozotocin (65 mg/kg, ip) and nicotinamide (90 mg/kg, ip). The diabetic rats were orally treated with polyherbal formulation (therapeutic dose, 28.71 mg/kg and 2X therapeutic dose, 57.42 mg/kg) and metformin (150 mg/kg), for 12 weeks. Serum glucose levels, body weight, histopathology of pancreas were used to evaluate the antidiabetic activity while the histopathological study of eye and protein content in lens were used to evaluate the protective effect of polyherbal formulation on cataract development. The progression of cataract in rat lens was observed with a slit-lamp biomicroscope. A daily treatment of polyherbal formulation for 12 weeks resulted in significant ($p<0.05$) decrease in serum glucose level, body weight and improved protein content in the lens. It also delayed the incidence of cataract in diabetic rats. The histopathological study showed that there was regeneration of pancreatic β-cells. The results observed with polyherbal formulation were found to be comparable to those obtained with metformin. Results suggested that the polyherbal formulation possessed antidiabetic activity and prevented cataract development.

Key words: Cataract, streptozotocin, nicotinamide, polyherbal, antidiabetic

Diabetes mellitus (DM) is a chronic disease characterized by disorders in carbohydrate, fat and protein metabolism[1]. The number of patients with DM is predicted to increase globally to 200 million within the next few years[2]. World Health Organization reports showed that 32 million people had diabetes in the year 2000 in India and now 422 million adults are living with diabetes according to the latest 2016 data[3,4].

The cause of significant mortality in diabetes is due to the development of secondary complications[5]. DM leads to the development of both microvascular complications like retinopathy, cataract, nephropathy, and macrovascular complications like atherosclerosis, high blood pressure, heart attack and stroke[6].

In the present study, the effect of a polyherbal formulation was studied on one of diabetic complications, the cataract. It is clouding of the crystalline lens of the eye or in its envelope[7]. As the extracellular glucose diffuses into the lens, it is the most affected body part in diabetes[8]. The mechanism of cataract involves generation of free oxygen radicals, which causes direct modification of the inner lens proteins, such as cross-linking, aggregation, precipitation contributing to yield opacity and leading to cataract[6]. Now a days cataract is the major cause of blindness and surgery is the only available solution[9]. The prevention of opacification or delays in the development of cataract remains a challenge[10].

Many anticataractogenic agents are available, but due to lack of success in patients, no drug has yet been approved for clinical use[11]. There are many herbal products and polyherbal formulations with known anticataract activity such as *Ocimum sanctum*, *Curcuma longa*, *Azadirachta indica* and Diabecon 400[12], Okudiabet[13], Diashis[14] and Glycoherb[15]. Hence, the present study was undertaken to evaluate

***Address for correspondence**
E-mail: malikailas@gmail.com

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one such polyherbal formulation composed of six different herbal extracts. The composition of polyherbal formulation is given in Table 1. This formulation available in the market has been proved to be effective in diabetes, but there is no data available whether this polyherbal formulation is effective in preventing diabetic complications. Therefore, an attempt was made to evaluate its effectiveness in delaying cataract development.

MATERIALS AND METHODS

Streptozotocin (STZ) was procured from Chemvenio, LIC, Gulbarga, India. Nicotinamide (NIC) was procured from Lasons India Ltd, Taloja, Raigad, India and the polyherbal formulation was supplied by Nisarga Biotech, Pvt. Ltd., Satara, India.

Experimental animals:

Forty male Wistar rats (180-200 g) were procured from Shri Venkateshwara Enterprises, Bangalore, India and were housed in the animal house of Satara College of Pharmacy, Satara, India at a temperature of 25±1° and humidity (45±5 %) with a 12:12 h day:night cycle. Animals were kept in polypropylene cages with a stainless steel lid with free access to standard pelleted diet and water *ad libitum*. The studies were carried out in the Pharmacology Department of Satara College of Pharmacy. The experimental protocol was approved by the Institutional Animal Ethics Committee of Satara College of Pharmacy, Satara (SCOP/IAEC/017/11-12) and it was carried out according to the Committee for the Purpose of Control and Supervision on Experiments on Animals guidelines for laboratory animal facility.

Acute toxicity studies:

Acute oral toxicity of the polyherbal formulation was carried out as per the guidelines set by the Organization for Economic Co-operation and Development revised draft guidelines 423. Healthy Wistar rats (3 animals/dose) of either sex were used in this study. Rats were fasted overnight and orally fed with the polyherbal formulation in increasing dose levels of 5, 50, 300, and 2000 mg/kg. These rats were observed for behavioural (alertness, restlessness, irritability and fearfulness), neurological (spontaneous activity, reactivity, touch response, pain response and gait) and autonomic (defecation and urination) symptoms continuously for 24 h. After a period of 24 h, the animals were observed for the next 14 d for mortality.

TABLE 1: COMPOSITION OF POLYHERBAL FORMULATION

Ingredients	Botanical name	Quantity
Guduchi	*Tinospora cardifolia*	35 mg
Dalchini	*Cinnamomum zeylanicum*	50 mg
Haridra	*Curcuma longa*	48 mg
Methika	*Trigonella foenum-graecum*	180 mg
Nimba	*Azadirachta indica*	4 mg
Maricha	*Piper nigrum*	2 mg
Excipient	-	qs

Induction of experimental diabetes:

Rats were fasted for 16 h and NIC (90 mg/kg) was administered through intraperitoneal route. After 15 min of administration of NIC, STZ (65 mg/kg, ip) was injected. STZ is capable of inducing fatal hypoglycaemia as a result of massive pancreatic insulin release, therefore, the rats were provided with 10 % glucose solution after 6 h of STZ administration for the next 24 h to prevent hypoglycaemia. Mortality or any other adverse effects were closely monitored. After allowing a week's time for the development and aggravation of diabetes, rats with moderate diabetes (i.e. blood glucose concentration >200 mg/dl) that exhibited glycosuria and hyperglycaemia were selected for the experiment[16].

Experimental design:

After inducing experimental diabetes, rats were divided into five groups, each comprising a minimum of eight rats. Carboxymethyl cellulose (CMC; 0.1 % w/v) in distilled water was used as the vehicle. Polyherbal formulation at doses of 28.71 and 57.42 mg/kg was suspended in vehicle and administered to treatment groups orally every day for 12 w. These 5 groups of rats consisted of normal control group, normal rats administered with 0.1 % CMC (1.25 ml/kg/day, po); diabetic control group, STZ-NIC-induced diabetic rats, administered with 0.1 % CMC (1.25 ml/kg/day, po); standard treated group, diabetic rats administered with metformin (150 mg/kg/day, po) for 12 w; therapeutic dose group, diabetic rats administered with a therapeutic dose of polyherbal formulation (28.71 mg/kg/day, po) for 12 w. The therapeutic dose was calculated from human dose of polyherbal formulation, which was 319 mg for an adult of 70 kg and converted it to animal dose based on no observable adverse effect level (NOAEL)[17,18]. Diabetic rats administered with 2X therapeutic dose of polyherbal formulation (57.42 mg/kg/day, po) for 12 w to check the effectiveness of a higher dose.

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Evaluation of antidiabetic activity:

Serum glucose level and body weight was measured weekly. The dosage was adjusted weekly according to any change in body weight to maintain similar dose per kg body weight of rat over the entire period of study for each group. The survived animals from each group after 12 w were euthanized by sodium pentobarbital, with a dose of 200 mg/kg injection followed by cervical dislocation and each rat was utilized to test for histopathology of pancreas, one eye was used for histopathological studies and other eye for protein determination[19].

Evaluation of cataract development:

Rats were lightly anaesthetized with ether for assessing cataract progression by observing the rat lens with a slit-lamp biomicroscope in Drishti Eye Hospital, Satara. Pupil of rats was dilated by instillation 2 to 3 drops of Tropicacyl Plus® (tropicamide 0.8 % and phenylephrine 5 %) in the conjuctival sac of each eye. Tropicacyl was added prior to ophthalmic examination[20]. Lenticular changes were observed in each month. The progression of cataract observed during slit lamp examination is mentioned as follows, as per grading scale[21]; stage 0: clear lenses and no vacuoles present; stage 1: vacuoles cover approximately one half of the surface of the anterior pole, forming a subcapsular cataract; stage 2: some vacuoles disappeared and cortex exhibits a hazy opacity; stage 3: a hazy cortex remained and dense nuclear opacity present; stage 4: a mature cataract, dense opacity in both cortex and nucleus.

Estimation of total protein of eye lens:

For estimation of total proteins, the eyes were enucleated and the lenses were dissected separately[22]. One dissected lens was homogenized in 4.0 ml deaerated phosphate buffer saline (pH 7.3) using a homogenizer. The lens homogenate was dialyzed for 24 h and centrifuged for 2 h at 4° at 13 500 rpm. Protein content of the supernatant was determined using Biuret method kit (Rackon diagnostics, India)[23].

Histopathology of pancreas and eye:

The pancreas and eyes of animals were removed and fixed in 10 % formalin. Then they were embedded in paraffin. Organ sectioning was carried out on a microtome, sections were stained with haematoxylin and eosin and observed for histopathological changes under microscope[21].

Statistical analysis:

Data were expressed as mean±SE (standard error). Statistical analysis was done by using one way analysis of variance followed by Tukey's Multiple Comparison Test by using GraphPad Prism software. $P<0.05$ was considered as the minimal level of statistical significance.

RESULTS AND DISCUSSION

Acute toxicity studies did not show any mortality up to 2000 mg/kg given as single oral administration. Hence, the study was carried out at the dose levels of 28.71 mg/kg and 57.42 mg/kg. Diabetes was induced in 40 rats using STZ (65 mg/kg, ip) and NIC (90 mg/kg, ip). All animals from normal control group were alive till the end of the experiment. About 50 % of diabetic rats died from the diabetic control group, 12.5 % of diabetic rats died from standard group treated with metformin while 25 % of diabetic rats died in both therapeutic and 2X therapeutic dose of polyherbal formulation-treated group. The mortality rate in metformin and polyherbal formulation-treated group was less as compared to diabetic control group.

The effect of polyherbal formulation as well as metformin treatment on the blood glucose concentration in control and experimental groups of rats is shown in Table 2. There was an increase in serum glucose level in diabetic control group after administration of STZ-NIC intraperitoneally. The serum glucose level decreased significantly ($p<0.05$) after oral administration of metformin (91.4 %), therapeutic (82.5 %) and 2X therapeutic dose (87.51 %) of polyherbal formulation when compared with diabetic control group.

Change in the body weights of all 5 groups of animals during the course of study is given in Table 3. There was significant ($p<0.05$) decrease in the body weight of diabetic rats from control group compared to that of normal rats. In diabetic control rats, the weight loss was about 51.85 % at the end of study. However, this significant weight loss was prevented in diabetic rats orally treated with metformin as well as polyherbal formulation. The weight loss was reduced in metformin-treated group approximately by 7 % whereas in the groups treated with therapeutic dose of polyherbal formulation and 2X therapeutic dose of polyherbal formulation, the weight loss reduction was found to be 2 and 3 %, respectively.

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TABLE 2: EFFECT OF METFORMIN AND POLYHERBAL FORMULATION TREATMENT ON SERUM GLUCOSE

Name of group	Treatment given	Time interval (weeks)			
		0	4	8	12
Normal control	0.1 % CMC (1.25 ml/kg)	103.7±3.99 (n=8)	106.5±4.13 (n=8)	106.6±2.8 (n=8)	107.9±3.6 (n=8)
Diabetic control	0.1 % CMC (1.25 ml/kg)	205.1±12.94# (n=8)	255.5±6.02# (n=6)	301±10.39# (n=5)	348.3±11.4# (n=4)
Standard	Metformin (150 mg/kg)	203.4±12.62 (n=8)	162.9±12.85 (n=8)	137.9±11.23† (n=7)	112.6±8.549† (n=7)
Therapeutic dose	Polyherbal formulation (28.71 mg/kg)	207.8±7.078 (n=8)	177±6.61† (n=7)	153.9±7.01† (n=7)	127.2±7.45† (n=6)
2X Therapeutic dose	Polyherbal formulation (57.42 mg/kg)	205.5±10.74 (n=8)	173.2±10.28† (n=7)	148.3±12.54 (n=6)	119.4±11.23† (n=6)

Values are expressed as mean±SEM (standard error of the mean) n is the number of animals. Serum glucose is given in mg/dl. Statistical analysis is one way ANOVA followed by Tukey's multiple comparison test. P<0.05 was considered statistically significant; #data compared with normal control; †data compared with diabetic control

TABLE 3: EFFECT OF METFORMIN AND POLYHERBAL FORMULATION TREATMENT ON BODY WEIGHT

Name of group	Treatment given	Time interval (weeks)			
		0	4	8	12
Normal control	0.1 % CMC (1.25 ml/kg)	204.5±3.13 (n=8)	215.9±2.41 (n=8)	228.3±2.71 (n=8)	242±2.62 (n=8)
Diabetic control	0.1 % CMC (1.25 ml/kg)	175.2±6.54# (n=8)	159.9±9.06# (n=6)	145.0±3.24# (n=5)	125.5±5.17# (n=4)
Standard control	Metformin (150 mg/kg)	170.3±5.88 (n=8)	188.3±4.53† (n=8)	201.7±2.36† (n=7)	218.4±2.58† (n=7)
Therapeutic dose	Polyherbal formulation (28.71 mg/kg)	166.1±4.4 (n=8)	172.0±4.43 (n=7)	188.9±5.25† (n=7)	203.1±9.05† (n=6)
2X Therapeutic dose	Polyherbal formulation (57.42 mg/kg)	172.8±5.04 (n=8)	180.3±5.6 (n=7)	196.5±7.81† (n=6)	211.4±10.04† (n=6)

Values are expressed as mean±SEM. Statistical analysis by one way ANOVA followed by Tukey's multiple comparison test. P<0.05 was considered statistically significant; #data compared with normal control; †data compared with diabetic control

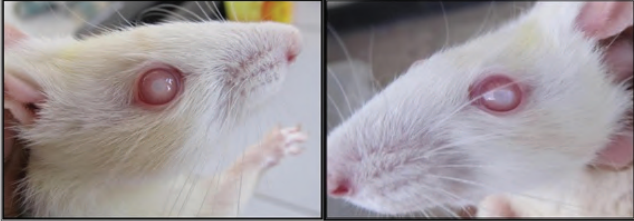
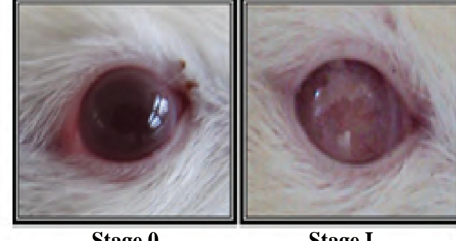
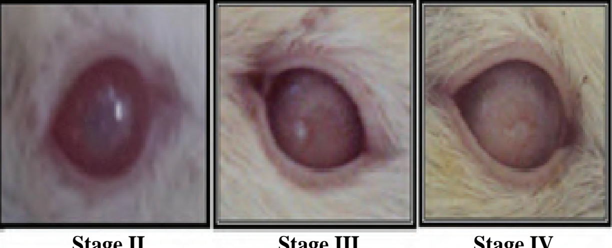


Fig. 1: Rats with cataract



Stage 0 **Stage I**



Stage II **Stage III** **Stage IV**

Fig. 2: Stages of cataract development

Cataracts were developed in the eyes of STZ-NIC-treated rats throughout the experimental period. The development of cataract and different stages of development are shown in the figs. 1 and 2. All lenses in the normal control group and standard group treated with metformin were clear throughout the study with no opacification. The onset of cataract in the diabetic control group was observed from the 3rd w of STZ-NIC injection. At the end of 12th w, 50 % lenses were in the 4th stage, 50 % lenses were in 3rd stage in the diabetic control group. Whereas onset of cataract in the polyherbal-treated group was observed from 10th w in case of therapeutic dose and from 12th w group in case of 2X therapeutic dose. The progression of cataract was slower in rats treated with therapeutic and 2X therapeutic dose of polyherbal formulation. At the end 12th w, 12.5 % of lenses of therapeutic dose were in

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the 2nd stage and 12.5 % lenses of 2X therapeutic dose were in the 1st stage as shown in the fig. 3.

The lens is made of mostly water and protein. Denaturation of proteins leads to lens opacification. Therefore, total protein content was analysed in all groups and the results are given in Table 4. The content of protein observed at the end of study in normal control rat was 1.02 g/dl, while in the diabetic control group it was 0.27 g/dl. Protein content was found to be significantly normal i.e. 0.91 g/dl in case of rats treated with metformin. While in rats treated with therapeutic dose and 2X therapeutic dose the protein content was 0.66 and 0.73 g/dl, respectively.

The results of histopathological examination of pancreas are shown in fig. 4. Fig. 4A showed normal acinar pattern and islet cells in normal control. There was no inflammation and fibrosis. Fig. 4B, showed the pancreas of rats treated with STZ-NIC. Number of islet cells was found to be decreased and also ratio of acinar to islet cell was not maintained. Deposits of a homogenous eosinophilic material largely occupying the islet and around blood vessels were seen. This could be a localized amyloidosis, which has been documented to occur in the pancreas in many diabetics.

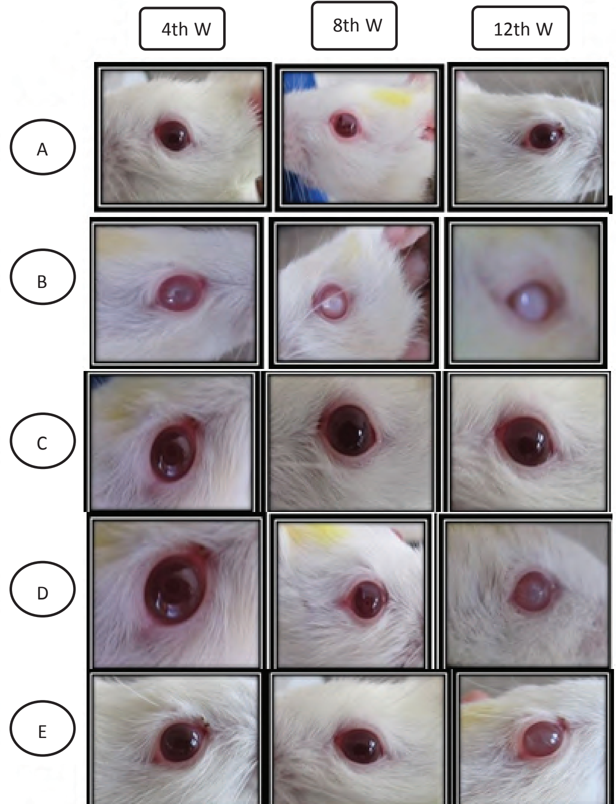

Fig. 3: Changes after treatment till 12th w
(A) Normal control; (B) diabetic control; (C) standard; (D) therapeutic dose; (E) 2X therapeutic dose

TABLE 4: ESTIMATION OF TOTAL PROTEIN FROM EYE LENS AT THE END OF STUDY

Name of group	Treatment given	Total protein (g/dl)
Normal control	0.1 % CMC (1.25 ml/kg/day, po)	1.0212±0.1333 (n=8)
Diabetic control	0.1 % CMC (1.25 ml/kg/day, po)	0.2741±0.0057# (n=4)
Standard	Metformin (150 mg/kg)	0.9100±0.05151† (n=7)
Therapeutic dose	Polyherbal formulation (28.71 mg/kg)	0.6554±0.04469c†# (n=6)
2X Therapeutic dose	Polyherbal formulation (57.42 mg/kg)	0.7292±0.03542† (n=6)

Values are expressed as mean±SEM. Statistical analysis was done by using one way ANOVA followed by Tukey's multiple comparison test. $P<0.05$ was considered statistically significant ([c]$p<0.05$); #data compared with normal control; †data compared with diabetic control

In case of pancreas treated with standard metformin, the acinar cells were found to be normal as shown in fig. 4C. The islets were present in adequate proportion and having normal cellularity of beta cells. There was no evidence of infiltration or fibrosis. The pancreas of rats treated with therapeutic dose of polyherbal formulation is shown in the fig. 4D. Presence of few islets of beta cells was observed. Islets were largely occupied by a uniform eosinophilic material and few atrophic cells. Eosinophilic materials also surround the blood vessel. There was slight regeneration of β cells. Fig. 4E, shows pancreas of rats treated with 2X therapeutic dose of polyherbal formulation. The acinar cells appeared to be normal. The islets were present with a large proportion and with smaller volume as compared to diabetic control. There was more regeneration of β cells as compared to therapeutic dose and further destruction of the remaining β cells in islet appeared to be stopped.

The results of histopathological examination of rat eyes are shown in fig. 5. Fig. 5A showed histopathology of normal control rat eye. The lens material showed deposition of fibrinoid material, and rest shows proteinaceous material. Retinal layers were normal. Fig. 5B, showed eye of rats treated with STZ-NIC. The lens material has hyalinised and fibrinoid material showing changes due to developed and developing cataract. Retinal layers showed degeneration and detachment. The histopathological section of eye treated with metformin showed normal lens material and retinal layers (fig. 5C). A nearly developing opacity and coagulation of lens material was observed in rats

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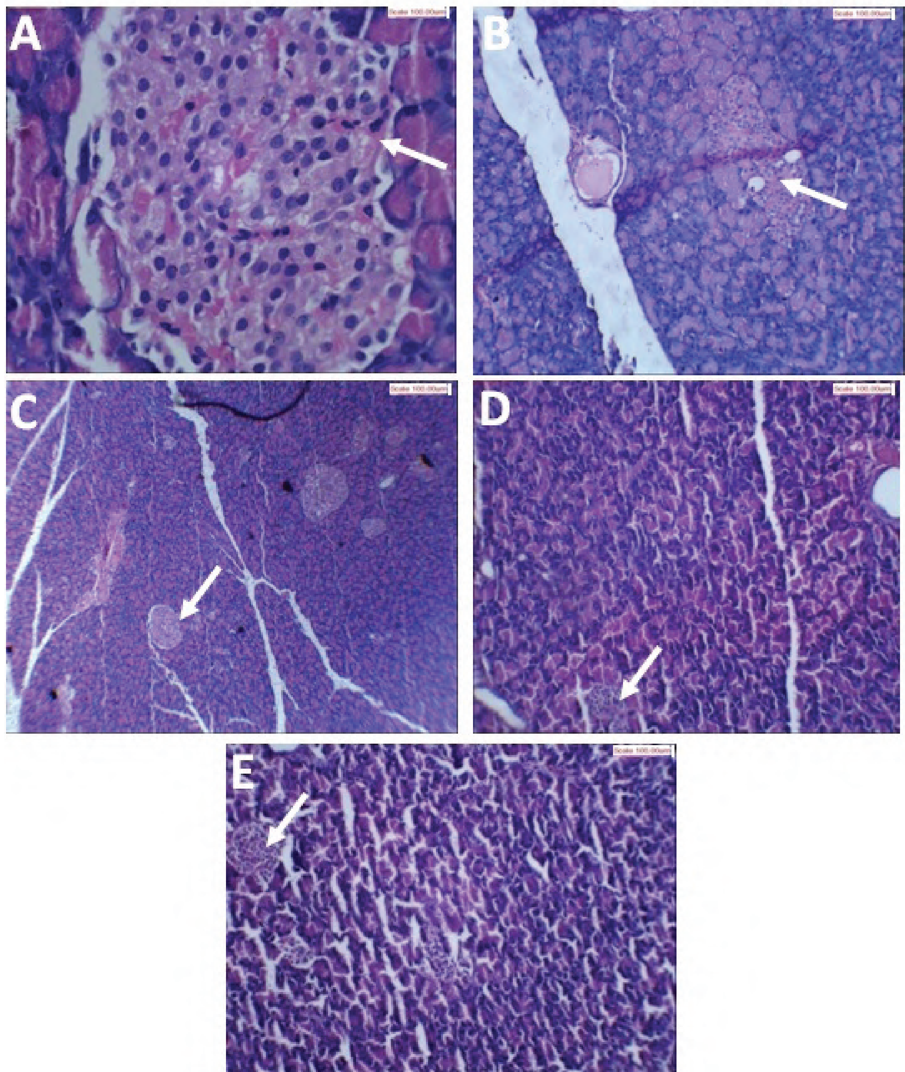

Fig. 4: Histopathological sections of pancreas of rats (scale 100 μm)
(A) Normal control; (B) diabetic control; (C) standard; (D) therapeutic dose; (E) 2X therapeutic dose. The arrow indicates the islet region of the section

treated with therapeutic dose of polyherbal formulation along with fibrosis of lens material (fig. 5D). The 2X therapeutic dose showed early changes of lens material along with hyalinization (fig. 5E).

In the present study, diabetes was induced by administration of STZ and NIC. STZ is an antibiotic and structurally is a glucosamine derivative of nitrosourea. It causes hyperglycaemia mainly by its direct cytotoxic action on the pancreatic beta cells. It also causes alkylation or breakage of DNA strands and a consequent increase in the activity of poly-ADP-ribose synthetase, leading to decrease in an enzyme in beta cells finally leading to energy deprivation and death of beta cells[24]. Here we had administered NIC along with STZ. The NIC is an antioxidant, which exerts protective effect on the cytotoxic action of STZ by scavenging free radicals and causes only minor damage to pancreatic beta cell mass producing type 2 diabetes.

The antidiabetic plant extracts may involve one or more compounds, which decreases blood glucose levels suggesting that the natural constituents could act synergistically to induce a hypoglycaemic effect[15]. Combining several medicinal herbs achieved extra therapeutic effect[25]. The polyherbal formulation used in the present study is composed of extract of six different herbs having different mechanism of action. The plant *guduchi* in formulation has antidiabetic activity, as it causes increased entry of glucose into the peripheral tissues and organs like liver. The oral administration of an aqueous root extract is reported to exert a significant reduction in blood glucose and brain lipids, increase in body weight. Also the alcohol extract of *guduchi* has preventive effect on the development

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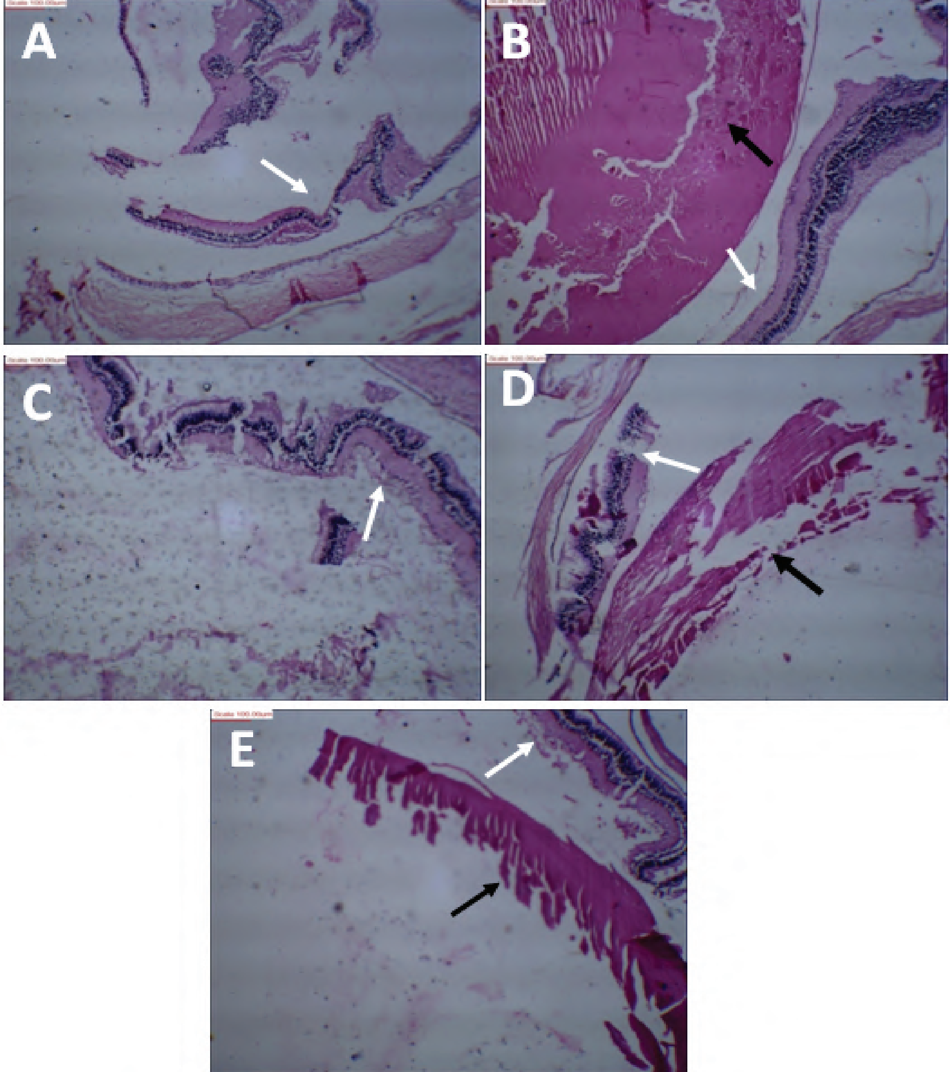

Fig. 5: Histopathological sections of eye (scale 100 µm)
(A) Normal control; (B) diabetic control; (C) standard; (D) therapeutic dose; (E) 2X therapeutic dose. The white arrow indicates retinal structure and black arrow indicates lens structure

of cataract in alloxan-induced diabetic rats[26]. Previous studies on cinnamon suggest it could affect protein phosphorylation dephosphorylation reactions in the intact adipocytes, which helps to reduce sugar level[27]. *Haridra* lowers blood sugar, increases glucose metabolism and potentiates insulin activity more than three fold. Several animal studies have demonstrated that curcumin present in *Haridra* can overcome insulin resistance and can delay cataract incidence in diabetic patients[28]. The studies on fenugreek showed that the hypoglycemic effects of fenugreek is due to presence of the amino acid 4-hydroxyisoleucine in fenugreek seeds. It causes insulin release in rat pancreatic islet cells[29]. The seeds may double the regeneration of β-cells in pancreas[30]. The leaves of fenugreek contain high content of lutein and prevent free radical formation. Hence it can be used as a good dietary source of lutein to protect and ameliorate cataract[31]. Neem blocks the action of epinephrine on glucose metabolism. It was found that chloroform extracts of Neem showed significant regeneration property of functional β-cells[12]. Piperine, a substance present in *Maricha* has been found to increase absorption of selenium, B-complex vitamins, beta-carotene, curcumin as well as other nutrients from food[32]. Thus effect of combination of all these extract in polyherbal formulation might decrease serum glucose levels, reduces destruction of β-cells and body weight, also improves protein contents and delays cataract.

Metformin was used as a standard drug in the current study. It acts principally by increasing insulin sensitivity and increase in the peripheral glucose utilization and decrease in hepatic glucose production. The UK Prospective Diabetes Study demonstrated

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a significant survival advantage for type 2 patients started on metformin as first-line therapy[33].

Treatment of polyherbal formulation showed significant decrease in serum glucose level. It may be due to effect of combination of all these extract in polyherbal formulation as discussed above. Metformin-treated animals showed rapid normalization of serum glucose as compared to polyherbal formulation-treated animals.

Polyherbal formulation treatment also prevented the loss in body weight as compared to diabetic control rats. It may be due to amelioration of glycaemic control and structural proteins synthesis[34]. Metformin has long been recognized as a suitable first-line agent for type 2 diabetes as it is the only oral hypoglycemic agent associated with no weight gain or even weight reduction[33].

Decrease in serum glucose level and weight loss prevented by the treatment with 2X therapeutic dose of polyherbal formulation is more as compared to therapeutic dose of polyherbal formulation. In the standard group cataract was not developed. This might be due to increased peripheral glucose utilization[34]. Treatment with polyherbal formulation delayed the progression of cataract. After induction of diabetes by STZ-NIC there was development of cataract in diabetic control group. Incidence of cataract was observed from 3rd w in diabetic control rats while in case of therapeutic dose it was at 10th w and in case of 2X therapeutic dose it was at 12th w. This suggested that the polyherbal formulation exerted protective action on cataract and also resulted in delaying progression of cataract. The 2X therapeutic dose showed greater delay as compared to therapeutic dose.

The lens is made of mostly water and protein. Crystallins are the major structural proteins in the lens accounting for up to 90 % of total soluble protein[35]. Protein denaturation has been considered to be the ultimate change that results in lens opacification. The treatment with polyherbal formulation improved the total protein content in eye lenses probably by preventing osmotic stress and subsequent leakage of proteins through the inhibition of aldose reductase (AR)[36]. This improvement was more in 2X therapeutic dose as compared to therapeutic dose. While metformin-treated group of rats showed more improved protein content as it is supposed to have an antioxidant property[37].

In diabetes there is destruction of pancreatic β-cells. Histopathological examination of pancreas supported presence of islet cells and few foci of regeneration of pancreatic β-cells. The regeneration of β-cells was comparatively more in rat pancreas treated with 2X therapeutic dose. In case of pancreas treated with standard metformin normal cellularity of beta cells was observed.

Histopathological study of eye lenses in normal group and metformin-treated group showed normal lens and retina while diabetic animals showed necrotic changes in lens and retina. The eye lenses-treated with therapeutic and 2X therapeutic dose showed early changes in the lens material. This also suggests that the polyherbal formulation can help in delaying cataract and it could be dose-dependent.

On the basis of these results, it can be concluded that a combination of glycemic control, regeneration of pancreatic β-cells, AR inhibition and antioxidant potential could be the possible mechanisms of polyherbal formulation in delaying the cataract. The polyherbal formulation is effective as antidiabetic and delays the progression of cataract in a dose-dependent manner. Moreover, further longer duration studies of polyherbal formulation in chronic models are necessary to assess a potent antidiabetic effect and its effect on diabetic complications.

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Home Automation Using Arduino And IoT

Pratiksha R. Patil[1], Chaitali T Ghadge[2]
Department of Electronics And Telecommunication
[1,2] BE, YSPM'S YASHODA TECHNICAL CAMPUS,SATARA

Abstract- *To improve the human living standard , reduces human efforts, energy and for the time saving smart home is the need of the times.The cost of project is low and the system offers a home security using Arduino microcontroller, Wi-Fi.Wi-fi is use foe access and the control appliances using Smart devices(smart phones,i-watch,PC,etc) application.*

The proposed system is being used to control the devices of people's daily needs by using nternet of things. We can use any electrical home devices like light,fan,fridge, tv,etc.for controlling with the help of web-browser, smart devices.

This system helps us to control different electrical devices such as light, fan, detection of gas leakage and vibration in home.

Keywords- Arduino ATmega328, ESP8266-01 vibration sensor, MQ6 gas sensor,light,fan,IOT

I. INTRODUCTION

The project aims at designing an advanced IOT based home automation system using web server and Wi-Fi technology. The devices can be switched ON/OFF using a smart devices. Automation is the need of hour.Wi-Fi is a technology that helps control the equipment of the house. Bluetooth and Zigbee are used to in most wireless networks [1]. In the system we will take ESP8266-01 Wi-Fi module in it we have put programming of Arduino uno to control electrical equipments.

The controlling device for the automation in the project is Arduino UNO. Arduino UNO reads the data,after that it decides the switching action of electrical devices which connected to it through Relays[2]. This proposed system is a combination of Android smart phone and embedded system which include Arduino Uno Board, Wi-Fi module and Relay circuit.[11] In our project , we used a Wi-Fi wireless technology to monitor the device. An android application is installed in a mobile device i.e android smart phone.With the help of this application we can control devices individually. The signal given from our smart devices received by Wi-Fi module through the cloud.

As per instructions given bythe user, the relay circuit switched ON/OFF the particular devices.The purpose of using Wi-Fi wireless technology is it provide a high range an.[3]

II. LITREATURE REVIEW

Through detailed study of "Home Automation Using Internet of Thing" proposed by Shopan Dey, Ayon Roy and Sandip Das,they explains, that they have used Raspberry pi module to connectESP8266-01 module to the internet.

Through this module they are controlling various device through web page and also through android application [1]. K. Venkatesan and Dr. U. Ramachandraiah explains in their paper Zigbee module in Arduino mega through which they are controlling devices. They have used various sensors for various purpose. Also they have provided real time notification, feedback on web-server in which customers can see what is happening in their home [2].

"Programmable Infrared Accessory Light Switch" by Warsuzarina Mat Jubadi and Normaziah Zulkifli explains how TV remote is used to control room light and other appliances. Here IR remote and one IR receiver is used and programmed it stores the frequency of the existing remote and use them directly to control appliances [3].

Twinkle Gondaliya proposed,A Survey on an Efficient IOT Based Smart Home [4] in that an efficient implementation for IoT for monitoring and automation system and it uses the portable devices as a user interface. Portable devices use for communicate with home automation network through an Internet gate, by means of low power communication protocols like zigbee, Wi-Fi etc.

This project aims at controlling home appliances via Smartphone using Wi-Fi as communication protocol and arduino uno. The user here will move directly with the system through a web-based interface over the web whereas home appliances like lights, fan etc. are remotely controlled through easy website.

"Vaibhav4, Vinay Dhakad Kunal1, Dhake Tushar2, Undegaonkar Pooja3, Zope Lodha5" explains "Smart Home Automation using IOT "[5].A System hardware is divided into three parts i.e. PCB, humidity sensor, and Arduino controller.

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Relay, LPT port, transistor, diode resister are integrated on PCB. They have connected two devices to the PCB i.e. fan and light. Humidity sensor is connected to Arduino .It will sense humidity and temperature as well.

Arduino and PCB are connected to PC..Arduino and PCB will interact with each other through PC. They have measured temperature and humidity. They have set time by which it continuously senses temperature and humidity. In the screenshot, it continuously senses temperature and humidity after every 5 seconds. ADVANTAGES (a) Adds Safety Through Appliance and Lighting Control (b) Secures Home Through web control Increases Convenience through Temperature Adjustment (c) Save time (d) Save money and increase convenience (e) Allow to appliances control when out of town.

H.Santhi,Gayathri.P,explains in paper [6] that the Home automation or automation of an office is done so with electronics and communication advancement.
Platforms based on cloud computing help to connect to the things surrounding everyone so that one can find it easy to access anything and everything at any time and place in a user friendly manner using custom defined portals.. The exciting opportunities yet to increase the connectivity and relationship of home devices automation purposes to the internet.

D.Maheshkumar explains in paper [8] about ideas, movements, technical approaches and considerations for strategic planning for the Internet of Things.

It was a simple review of research in IoT models, privacy issues and considerations for businesses to include in their way forward for IoT. Attempts are being made to harness the seismic shift caused by the IoT movement. While unification or standardization is lacking it seems like it may be left to economic competition on what moves forward.
This paper was a very small fraction of what IoT literature provides and was meant to educate individuals as it has done for the author in order to initiate discussions at their organizations. Prof .S A Jain.Stevan Maineka, Pranali Nimgade,explains in paper [9] The Internet of Things (IoT) is an atmosphere in which objects, animals or people are make available with distinct identifiers 'Internet of Things' defines a number of skills and research disciplines that allow the Internet to reach into the real world of physical objects. Technologies like short-range wireless communications, RFID, ad hoc and wireless sensor networks (WSNs) which is the part of Internet of Things (IoT).. This paper describes the concept of WSN, IoT and architecture of Home Automation.
Arun Cyril Jose1 and Reza Malekian2 in paper [10] Various Home Automation Methodologies Analyzed from a Security Standpoint and Challenges in Home Automation Security. Various home automation technologies considered in this work include context-aware home automation systems, central controller-based home automation systems, Bluetooth-based home automation systems, Global System for Mobile communication or mobile-based home automation systems, Short Messaging Service-based home automation systems, General Packet Radio Service-based home automation systems, Dual Tone Multi Frequency-based home automation systems, and Internet-based home automation systems.

III. PROBLEM STATEMENT

When people are pursuing ever-growing high quality of their lives today.This leads to more and more facilities and home appliances include into their building.How to control and manage these versatile facilities and appliances in a house?

When we are outside of the home and gas leakage will happen ,and also whenever earthquake will occur,then what shall we do?

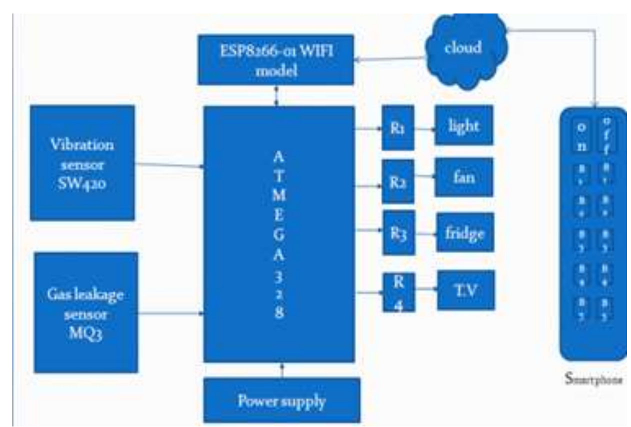
The main aim of this project “automatic home” to minimize the cost & loss electricity and also man power to manually on-off the home appliances.

IV. PROPOSED WORK

System architecture: This system consists of Arduino controller ATMEGA 328p,powersupply,vibrationsensor,gas leakage sensor,relays,light,fan and smart phone.

The main aim of this project is to build a smart home device which can be used to control the home appliances via internet.

BLOCK DIAGRAM:



FLOW CHART OF WORKING DEVICES:

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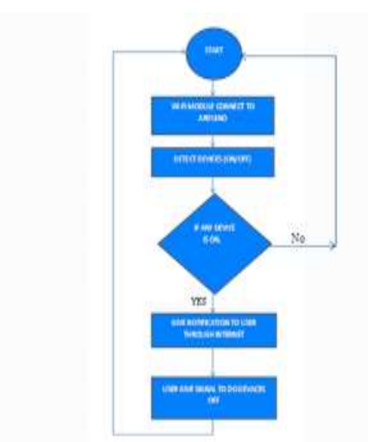

System working:

vibration sensor and gas leakage sensor connected to the Arduino controller atmega328p and we also used four relays for the light, fan, and by using other two relays we will give OR gate connection to the main switch. When lights, fan and gas is leaked at home, you will get a notification on the smart phone. The device can also connected to an Android App which you can develop on your own using some applications like MIT App inventor etc.

By using this app, we will be able to monitor and control the home appliances from any part of the world with ease. when you get a notification, you can turn off the button in your phone. User give a signal to do devices off. this signal goel to the wifi model through cloud and it will sent to the atmega328p.whenever there is an earthquake, the notification will go on the user's phone, so the main switch will be switched off and short circuits will not happen.

Proposed System Implementation:

1. Start
2. System will be initialized.
3. Initially all control devices will be OFF after power-on.
4. Wi-Fi module will be initialized.
5. Open mobile application on Android smart phone.
6. Establish connection between Wi-Fi module and mobile application on Android Smart phone.
7. Waiting for control command to be received from Android mobile application.
8. Send control command (ON/OFF device) from Android mobile application.
9. Check received control command format.
10. If received command is "ON" then turn ON the particular Device.
11. If received command is "OFF" then turn OFF the particular Device.
12. stop.

ADVANTAGES:

1. Devices can be controlled from long distance.
2. It can be easily situated homes.
3. It can use everyone .only we have knowledge of the text.
4. Format of the text is easy to understand.

DISADVANTAGES:

The system is network dependent. Hence network congestion can reduce the reliability of the system.

V. CONCLUSION

When you are out of the house, you cannot turn off the electrical appliances in the house.Also there is a possibility of fire in the house due to gas leakage and earthquake. So, we are doing "IOT based Home Automation using Arduino Uno" to avoid all this.

Due to this project, when we are out of the house we can turn off electrical appliances in the house. The main switch will be switched off when the gas leakage goes beyond the specified limit, due to the gas sensor. When an earthquake or a big quake occurs, there will be vibration sensor in the house and the main switch will be switched off.

Benefit of this project is, power utilities will not continue unnecessarily. Electricity will be saved. The harm caused by gas leakage can be prevented by the gas sensor. Due to earthquake there will be a short circuit that will be avoided. This project will make people's lifestyle comfortable to some extent.

FUTURE SCOPE

Reducing the time delay to turn on and off of an appliance in the home. Adding speech identification to the system using automatic smart phone detection through Wi-fi such that it will operate the loads automatically when it is in range. Expansion of range of Wi-Fi such that one can operate allowed long distance through smart phone.

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Original Article

Design, Development and Evaluation of Self Nanoemulsifying Drug Delivery System of Garlic Oil using Capryol PGMC

Priyanka Sangar[1], Bandgar Sandip[1,*], Shelake Sardar[2], Patil Pravin[1], Bhagwat Durgacharan[3], Patil Shitalkumar[1]

[1]Department of Pharmaceutics, Ashokrao Mane College of Pharmacy, Peth-Vadgaon, Kolhapur, Maharashtra, INDIA.
[2]Department of Pharmaceutics, Ashokrao Mane Institute of Pharmacy, Ambap, Maharashtra, INDIA.
[3]Department of Pharmaceutics, Bharati Vidyapeeth College of Pharmacy, Kolhapur, Maharashtra, INDIA.

ABSTRACT

Introduction: At present days there was considerable attention has been taken to develop lipid based pharmaceutical preparation which improves solubility as well as permeability leads to improve oral bioavailability of poorly water soluble drug with a system known as self nano-emulsifying drug delivery system. **Materials and Methods:** The SNEDDS of garlic oil was prepared by using oleic acid as oil, capryol PGMC as a surfactant and ethanol as a co-surfactant, as the garlic oil shows better solubility in these excipients which is find out by constructing pseudo-ternary phase diagram. The Km = 3 was selected for the preparation of SNEDDS of garlic oil because it shows better nanoemulsion region as compared to Km = 1 and 2. **Discussion:** The formulated SNEDDS of garlic oil was evaluated for physical characterization, thermodynamic stability, rheology study, globule size and zeta potential, dispersibility study, cloud point determination, % transmittance, drug content, FTIR study and *in vitro* drug release study. Three batches of SNEDDS of garlic oil was formulated using Km value 3 which cover maximum nanoemulsion region, containing oleic acid (solubility 57.53 ± 0.45), Capryol PGMC (solubility 59.80 ± 0.82) and ethanol (solubility 49.83 ± 0.30). Based on the compatibility study, optimum globule size (177.2 nm), minimum polydispersity (0.386), higher drug content (90.89 ± 0.68) and higher drug release (98.85%), batch F2 was optimized. **Conclusion:** The bioavailability problem can be overcome by the Self nano- emulsifying drug delivery system, which presents the more drug in solubilized form in the body as compared with other conventional drug delivery systems.

Key words: Self Nanoemulsifying Drug Delivery System, Garlic oil, Pseudo ternary phase diagram, Capryol PGMC, poorly water soluble drug.

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Correspondence:
Mr. Sandip Akaram Bandgar,
Department of Pharmaceutics, Ashokrao Mane College of Pharmacy, Peth-Vadgaon, Kolhapur, Maharashtra-416112, INDIA.
Phone: +91 8600009719
E-mail: bandgarsandip21@gmail.com

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INTRODUCTION

Garlic, botanically known as *Allium sativum* Linn. a member of Liliaceae family is one of the earliest documented example of plants employed for the treatment of diseases and maintenance of health.[1] Garlic oil is best known for its number of medicinal values such as anti-atherosclerosis, blood lipid and sugar modulation, antifungal, antimicrobial, anti-thrombolic, cardiovascular disease treatment and stimulation of immune system.[2] However, the application of garlic oil in the food industry is limited due to its volatility, strong odour, insolubility in water and low physicochemical stability.[3] To overcome these problems various methods are listed in the literature which include incorporation of hydrophilic excipients, solid dispersion, micellar solubilization, microemulsion etc. But in recent years considerable attention has been made to develop lipid based pharmaceutical preparation as it improves not only solubility but also permeability which leads to improve oral bioavailability of poorly water soluble

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drugs, such a system is known as Self Nanoemulsifying Drug Delivery System (SNEDDS).[4]

Self Nanoemulsifying Drug Delivery Systems (SNEDDS) are regarded as anhydrous forms of the nanoemulsion. SNEDDS are homogenous liquid mixtures consisting of drug, natural or synthetic oil, surfactant and co-surfactant that have a rival ability of spontaneously forming fine oil-in-water (O/W) nano-emulsions of size about 200 nm or less, upon dilution with water. These preparations are thermodynamically stable and transparent or translucent system. Nano-sized dispersion of nanoemulsion was stabilized by the addition of surfactants and co-surfactants. SNEDDS are also known as nanoemulsion, miniemulsion, ultrafine emulsion or submicron emulsion. These systems were formulated mainly by using medium chain triglycerides, oils and non-ionic surfactant, which is important in oral ingestion. SNEDDS are one of the stable nanoemulsion and it provides a large interfacial area for partitioning of drug between oil and aqueous phase, thereby improves the rate of drug dissolution and increases bioavailability of the drug formulation. SNEDDS are the most preferred drug delivery system due to their stability, practicability of easy oral administration and ability to enhance drug self emulsification inside the gut.[5,6]

Thus, utilizing SNEDDS as a promising technology to overcome the problems of low bioavailability leads to develop a drug with improved solubility as well as improved physiochemical stability.[7] Hence, SNEDDS of garlic oil will control different aspects of drug efficacy such as pharmacokinetics, bioavailability, targeted delivery, non-specific toxicity and immunogenicity and will be beneficial as suitable dosage form which results in better patience compliance and improved therapeutics.[8,9]

MATERIALS AND METHODS

Garlic oil (Sanket Enterprises, Mumbai), Oleic acid (Molychem, Mumbai), Capryol PGMC (Gattefosse, France), Ethanol and Methanol (S. D. fine Chemicals, Mumbai). All other materials or chemicals used were of analytical grade.

Selection and screening of drug components

For the selection of suitable components with good solubilizing capacity for garlic oil, saturation solubility of garlic oil was examined in various oils (oleic acid, cotton seed oil, almond oil, castor oil), surfactant (Capryol PGMC, Labrafac PG, tween 20, span 80, cremophore EL) and co-surfactants (Ethanol, propylene glycol, PEG 200, glycerol). In this solubility study the excess amount of drug i.e. garlic oil was added into screw caped glass vials containing two ml of each excipients followed by sealed vials. The sealed vials were kept in sonicator for 2 h. after that the mixture was kept in water bath at 40°C for 24 h and then these vials were centrifuged at 15000 rpm for 30 min. The samples were collected and filtered using a membrane filter (0.45 micro meter). The filtrate was suitably diluted with methanol and drug concentration was obtained by using UV Visible spectrophotometer.[10]

Construction of pseudo ternary phase diagram

The pseudo ternary phase diagram was constructed without garlic oil to recognize the maximum self-emulsifying domain existence and to specify the optimal ratio of oil, surfactant and co-surfactant for the SNEDDS formulations. The pseudo ternary phase diagrams were constructed by drop wise addition of distilled water to homogeneous liquid mixture of oil, surfactant and co-surfactant, at ambient temperature by water titration method.

From result of solubility studies and screening of solubility of excipient: Oleic acid, Capryol PGMC and ethanol were selected as oil, surfactant and co-surfactant. The mixture of oil and surfactant / co-surfactant (S/CoS) i.e S_{mix} at certain weight ratio were diluted with water in drop wise addition. Surfactant and co-surfactant mixture were mixed in different weight ratio at different Km value 1, 2, 3 ratio i.e. 1:1, 2:1, 3:1 (w/w). The oil and S_{mix} were mixed at ratio of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 Figure 2, 3 and 4. Slow titration with aqueous phase was done to each ratio of oil and S_{mix} and visual observation was carried out for transparency and flowability of nanoemulsion. The mixtures were examined for turbidity to transparency. Clear and isotropic mixtures were deemed to be within Nano emulsion region. On the other hand, the emulsion with coarse droplets or temporary emulsion exhibiting coalescence or creaming on terminating stirring was considered "bad". All the tests were performed in triplicate.[11,12]

Preparation of liquid SNEDDS

The phase diagram was constructed at different Km values. The Km value at which nano-emulsion region obtained was selected for further studies. Three formulations were selected from this nano- emulsion region.

Oil, surfactant and co-surfactant were accurately weighed and mixed by gentle stirring. Based on solubility, formulation amount of garlic oil (100mg) was dispersed into mixture of oil and surfactant and co-surfactant. All the components were mixed by gentle stirring on

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Table 1: Composition of selected formulation.

Batch code	Drug (mg)	Smix (ml)	Oil (ml)	Water (ml)
Garlic F1	100	30	10	60
Garlic F2	100	40	10	50
Garlic F3	100	50	10	40

magnetic stirrer until garlic oil was completely dissolved. Mixture was sealed in glass vial and stored at room temperature for further study.[13] The composition of selected formulations showed in Table 1 and Figure 6.

Evaluation of SNEDDS[14-16]

Physical characterization

The organoleptic properties of the SNEDDS such as, color, odor and physical state were checked by visual observation.

Thermodynamic stability study

The thermodynamic stability of lipid based formulation can be adversely affected by precipitation of the drug in the excipients matrix. This can be also lead to phase separation of the excipients affecting not only formulation performance as well as visual functioning. The thermodynamic stability study was based on following three tests:

Heating and cooling cycle

Three heating/cooling cycles between 4°C and 40°C with storage at each temperature for not less than 24 h. The resultant formulations were evaluated for their thermodynamic instability like precipitation and phase separation. The formulation which qualifies this test was subjected to further study.

Centrifugation study

The prepared formulations were centrifuged using laboratory centrifuge at 5000 rpm for 30 min. The resultant formulations were then determined for any instability problem, such as phase separation, cracking or creaming. A formulation which qualifies this test subjected for further study.

Freeze thaw cycle

To determine the stability of SNEDDS freeze thawing was employed. The prepared formulations were subjected to three freeze thaw cycles, which included freezing at –4°C for 24 h followed by thawing at 40°C for 24 h. Then centrifugation was performed at 3000 rpm for 10 min. Then the tested formulations were observed for phase separation.

Rheological study

The viscosity of the prepared formulations was determined by using Brookfield viscometer which determines the consistency of nano-emulsion formulation. 1ml of each prepared formulations were diluted 10 times with distilled water and then viscosity was measured using Brookfield viscometer and assessed visually for any phase separation.

Globule size and zeta potential determination

Droplet size of SNEDDS was determined by photon correlation spectroscopy that analyses the fluctuations in light scattering due to Brownian motion of the particle, using a Zetasizer. The zeta potential of the SNEDDS should be evaluated as it may further give an idea of the colloidal stability. Both these tests were carried out by using Nanoparticle analyzer sz-100 (Horiba Scientific, Japan).

Dispersibility test (Assessment of self emulsification)

The efficiency of self- emulsification of oral nanoemulsion is determined by using a standard USP XXII dissolution apparatus II. 1ml of each formulation is added to 500 ml of water at 37±0.5°C. The stainless steel dissolution paddle rotating at 50 RPM provided gentle agitation. The emulsification time assessed visually.

Percent transmittance

The percent transmittance of the prepared formulations were measured using UV Visible double beam spectrophotometer or Single Beam Spectrophotometer using distilled water as blank at suitable wavelength. For this study 1ml of each prepared formulations were diluted to 100 ml of distilled water and observed for any turbidity and % transmittance was observed by using UV-visible spectrophotometer (Shimadzu UV 1800) against distilled water at suitable wavelength.

Cloud point determination

The prepared formulations were diluted with distilled water in the ratio 1:250, placed in water bath and its temperature was increased gradually. Cloud point was measured at the temperature at which there was a sudden appearance of cloudiness occurred.

Drug content

The total amount of drug in the formulation was analyzed by dissolving the formulation in 10 ml of

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methanol. This solution was vortexed for 10 min in vortex mixture. The mixture was centrifuged at 15,000 rpm for 10 min. Then the supernatant was filtered through Whatman filter paper. The concentration of garlic oil was analyzed spectrophotometrically at 306 nm.

FTIR Study

The prepared formulations were analyzed by Fourier Transform infrared spectroscopy (UV Agilent Technology) to characterize the probable structural modification produced. The sample was analyzed in the region of 4000 and 400 cm^{-1} and then sample or mixture kept into sample holder for analysis.

In vitro drug release study

In vitro dissolution studies of prepared formulations were carried out. The prepared formulations were filled in hard gelatin capsule. *In vitro* drug release profile of garlic oil from SNEDDS was assessed using USP dissolution testing apparatus I (basket type) at 50 rpm with 900 ml 0.05 M NaCl of pH 1.5 as dissolution medium. Temperature was set at 37. 0± 0.5°C and sampling interval were fixed at 5, 10, 15, 20, 25, 30 min.1ml of sample withdraw at each time interval and replaced with 1ml fresh 0.05M NaCl of pH 1.5 solution. The solution was immediately filtered through whatman filter paper and the filtrate was diluted with dissolution medium up to 10ml and evaluated for the drug content using UV-Visible spectrophotometric method at 306 nm.[17]

RESULTS AND DISCUSSION

From the solubility study oleic acid was selected as oil, capryol PGMC as surfactant and ethanol as co-surfactant, as the garlic oil shows more solubility than the other components which were shown in Figure 1.

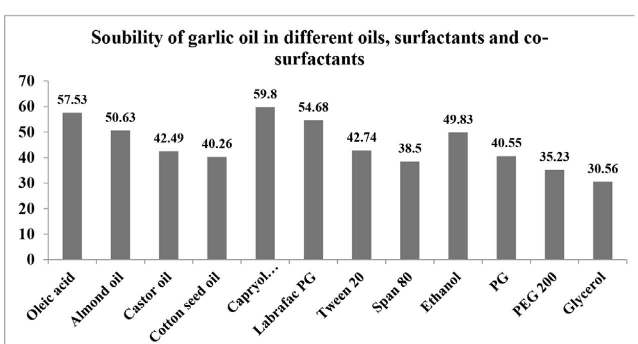

Figure 1: Solubility of garlic oil in different oils, surfactants and co- surfactants.

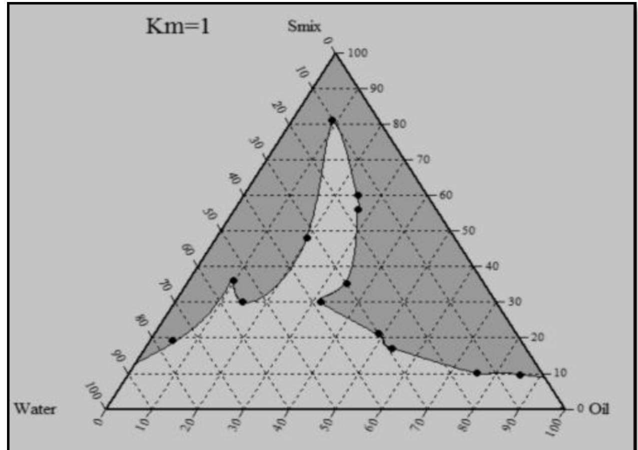

Figure 2: Ternary phase diagram of Oleic acid, Capryol PGMC, Ethanol at Km=1.

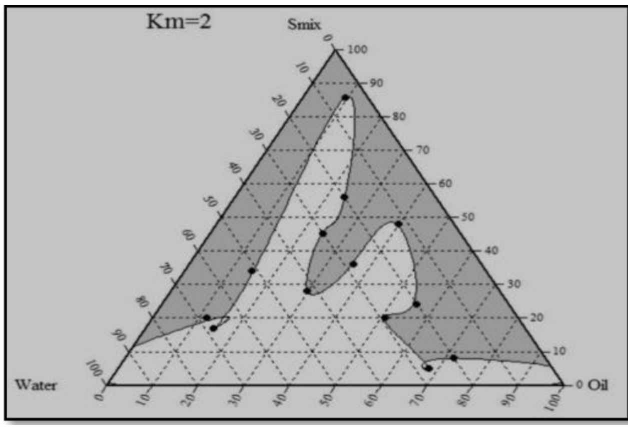

Figure 3: Ternary phase diagram of Oleic acid, Capryol PGMC, Ethanol at Km=2.

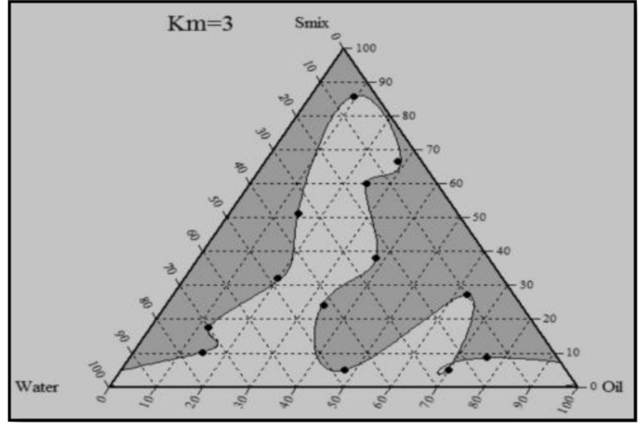

Figure 4: Ternary phase diagram of Oleic acid, Capryol PGMC, Ethanol at Km=3.

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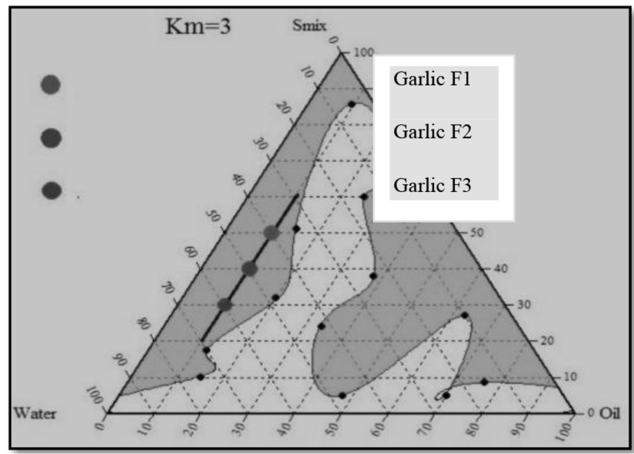

Figure 5: Selected composition of formulations Garlic F1 to Garlic F3.

Figure 6: Formulated batches of SNEDDS of Garlic oil.

Construction of pseudo-ternary phase diagram

Preparation of liquid Self Nano-emulsifying Drug Delivery System

Oleic acid-Capryol PGMC-Ethanol-Water based system selected at final Pseudoternary phase diagram of various surfactants and co-surfactant weight ratio was constructed and system of highest water absorption (highest nano emulsion region) selected for formulation. The phase diagram at Km value 3 showed better nano-emulsion existence region than 1 and 2.

Three formulations were selected from phase diagram at Km value 3, named as Garlic F1, Garlic F2, Garlic F3, as shown in Figure 5. Quantitative unit compositions of selected formulation of SNEDDS were presented in Table 2.

Evaluation of prepared SNEDDS

Physical characterization

The physical characterization of formulated batches was shown in Table 3.

Table 2: Composition of selected formulations.

Batch	Drug (mg)	Smix (ml)	Oil (ml)	Water (ml)
F1	100	30	10	60
F2	100	40	10	50
F3	100	50	10	40

Table 3: Physical characterization of formulated batches.

Sr. No.	Parameters	Result
F1	Physical state	Liquid
F2	Color	Light yellow
F3	Taste	Characteristic

Table 4: Thermodynamic stability study of formulated batches.

Batch	Heating cooling cycles	Centrifugation test	Freeze thaw cycles
Garlic F1	+	+	+
Garlic F2	+	+	+
Garlic F3	+	+	+

Thermodynamic stability study

Thermodynamic stability of SNEDDS was essential to its performance, which can be affected by precipitation of the drug. In addition the formulation having poor physical stability can affects the formulation performance and it also leads to phase separation. Hence thermodynamic stability studies were performed by performing heating cooling cycle, centrifugation test and freeze thaw cycle, it was observed that formulation passed the heating cooling cycle test, hence further exposed to centrifugation test then it was taken for freeze thaw stress test. After freeze thaw stress test it was found that all three formulations showed good stability with no phase separation, creaming or cracking were showed in Table 4.

Rheological study

The rheological properties of the prepared formulations were evaluated by Brookfield viscometer. This viscosities determination confirm the system is o/w or w/o. If system has low viscosity then it is o/w and high viscosity then w/o. Viscosity of prepared batches was determined by diluting 1 ml sample of each batch with 10 ml and 100 ml of distilled water by using Brookfield viscometer. The obtained results were showed in Table 5.

Globule size and zeta potential determination

The globule size of the emulsion is a crucial factor of self nano-emulsification performance because it deter-

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mines the rate and extends of drug release as well as drug absorption. Also, smaller particle size of the emulsion droplets may lead to more rapid absorption and improve the bioavailability.

The globule size and zeta potential determined using Nanoparticle analyzer sz-100. The average globule size was taken into consideration. Table 6 shows the particle size, zeta potential and PDI of formulated batches of garlic oil SNEDDS diluted with water. The average particle size obtained from optimized batch Garlic F2 of SNEDDS formulation of garlic oil was found to be 177.2 nm, zeta potential -25 mv and polydispersity index was found to be 0.386 Figure 7 and 8. Zeta potential is the another property that was assessed for increased absorption of SNEDDS is the charge of oil droplets which is usually found to be negative due to the presence of free fatty acid. These results indicate that the optimal garlic oil SNEDDS formulation produced clear nano emulsion with nanometric size.

Dispersibility test (Assessment of Self Emulsification)

Emulsification time is a major parameter that helps in the determination of emulsification rate of SNEDDS. Oil is a major factor that affect relatively because when it present in high concentration, it prevent penetration of water. While hydrophilic compound such as surfactant and co-surfactant helps in dispersion and so enhance the emulsification rate. The efficiency of self- emulsification could be estimated primarily by determining the rate of oil droplets of SNEDDS formulation dispersed quickly and completely when subjected to aqueous dilution under agitation. The self- emulsification time of prepared formulation of SNEDDS were show in Table 7.

Percent transmittance

The results of % transmittance were shown in Table 8. The clarity of prepared nano emulsion was checked by transparency, measured in terms of transmittance. SNEDDS forms o/w nano emulsion since water is external phase. Formulation Garlic F2 has 97.50 % transmittance. The result indicates good clarity of emulsion Table 8.

Cloud point determination Cloud point of prepared nanoemulsion was found to be higher than 80°C, which indicate that nanoemulsion will be stable at physiological temperature without risk of phase separation. The obtained results were showed in Table 9.

Drug content The drug content of the prepared formulations was shown in Table 10.

Table 5: Viscosity determination of formulated batches.

Sr. No.	Batch	Viscosity Cp	
		10 ml dilution	100 ml dilution
1	Garlic F1	0.5467	0.3589
2	Garlic F2	0.4043	0.3467
3	Garlic F3	0.5689	0.3654

Table 6: Globule size, Zeta potential and PDI of prepared formulations.

Sr. No.	Batch	Average Particle size (Droplet size /Globule size	Zeta potential	Poly-dispersity index (PDI)
1	Garlic F1	185.00 nm	-20 mv	0.567
2	Garlic F2	177.2 nm	-25 mv	0. 386
3	Garlic F3	193.90 nm	-18 mv	0.690

Table 7: Self-emulsification time of prepared formulation.

Sr. No.	Batch	Emulsification time (sec)
1	Garlic F1	59.83 ± 0.76
2	Garlic F2	70.30 ± 0.49
3	Garlic F3	60.36 ± 0.28

Table 8: % Transmittance of prepared formulations.

Sr. No.	Batch	% Transmittance
1	Garlic F1	92.71 ± 0.25
2	Garlic F2	97.50 ± 0.40
3	Garlic F3	95.33 ± 0.41

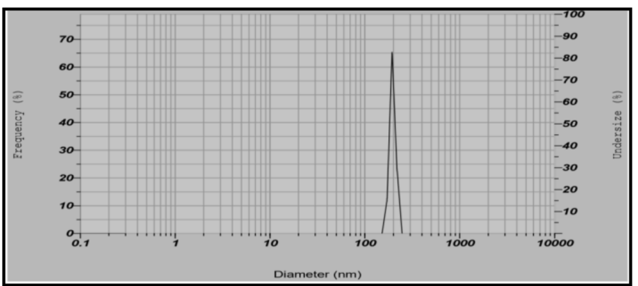

Figure 7: Globule size analysis of optimized batch Garlic F2.

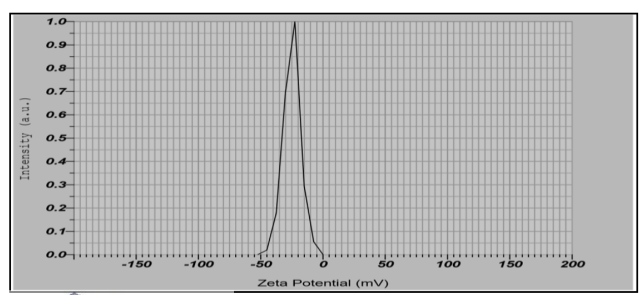

Figure 8: Zeta potential of optimized batch Garlic F2.

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Table 9: Cloud point determination of prepared formulation

Sr. No.	Batch	Cloud point
1	Garlic F1	More than 80°C
2	Garlic F2	More than 95°C
3	Garlic F3	More than 90°C

Table 10: Drug content of prepared formulations.

Sr. No.	Batch	% Drug content
1	Garlic F1	75.05 ± 0.55
2	Garlic F2	90.89 ± 0.68
3	Garlic F3	67.98 ± 0.75

Table 11: % Drug release of prepared formulations.

Sr. No.	Batch	% Drug release
1	Garlic F1	85.78
2	Garlic F2	98.85
3	Garlic F3	90.78

FTIR study

Drug and formulation has shown no any difference in spectra indicate drug is intact in the formulation which was shown in Figure 9.

In vitro drug release

In vitro drug release study of prepared formulations of garlic oil SNEDDS was performed in 0.05 M NaCl of pH 1.5. The % drug release was shown in Table 11 and Figure 10.

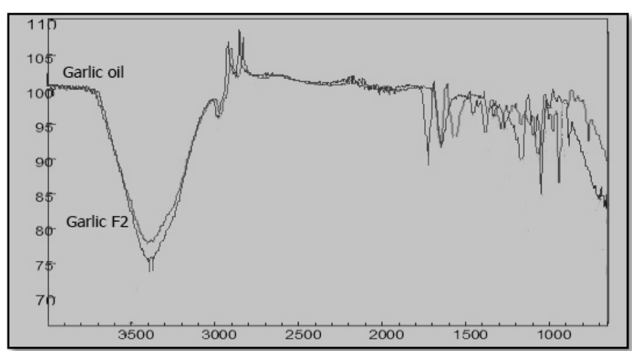

Figure 9: FTIR Spectra of garlic oil and optimized batch F2.

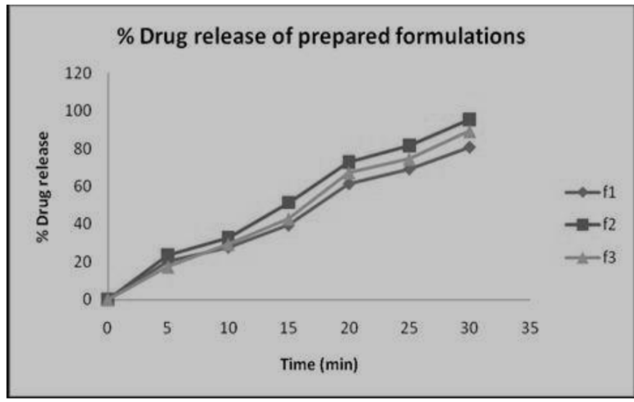

Figure 10: % Drug release of prepared formulations.

CONCLUSION

In this study, liquid SNEDDS was formulated by using capryol PGMC as surfactant. From this study, it was concluded that the prepared liquid SNEDDS was thermo dynamically stable with good self-emulsification efficiency, improved dissolution rate and having globule size in the nanometric range which may be physiologically stable. The SNEDDS with relatively high drug content was prepared which self-emulsified easily with mean emulsion droplet size of 177.2 nm. Thermodynamic stability study and cloud point study confirmed that the SNEDDS had no dilution effect and was stable without any precipitation of drug and without any change in emulsion droplet size.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

FTIR: Fourier Transform Infrared Spectrometer; **SNEDDS:** Self Nanoemulsifying Drug Delivery System; **RPM:** Revolutions per Minute; **PGMC:** Propylene Glycol Monocaprylate.

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PICTORIAL ABSTRACT

Problems associate with Garlic oil:
Volatility, Strong odour, Insolubility in water, Low physicochemical stability

SUMMARY

In this present work, liquid SNEDDS of garlic oil was formulated by using capryol PGMC as surfactant. From this study, it was observed that the prepared liquid SNEDDS of garlic oil was thermodynamically stable with good self-emulsification efficiency, improved dissolution rate and having globule size in the nanometric range which may be physiologically stable. The garlic oil shows better solubility in oleic acid (oil), capryol PGMC (surfactant) and ethanol (co-surfactant) which was find out by constructing pseudo-ternary phase diagram. The Km=3 was selected for the preparation of SNEDDS of garlic oil because it shows better nanoemulsion region as compared to Km=1 and 2. The SNEDDS with relatively high drug content was prepared with mean emulsion droplet size of 177.2 nm. Thermodynamic stability study and cloud point study confirmed that the SNEDDS had no dilution effect and was stable without any precipitation of drug and without any change in emulsion droplet size.

About Authors

Ms. Priyanka C. Sangar, has completed B Pharm and M Pharm in Pharmaceutics from Ashokrao Mane College of Pharmacy, Peth Vadgaon in 2018. Currently, he is working as Assistant Professor at Yashoda Technical Campus, Satara.

Mr. Sandip A. Bandgar, has completed B Pharm in 2004 and M. Pharm (Biopharmaceutics) in 2008 from Government College of Pharmacy, Karad and pursuing his Ph.D from Shivaji University, Kolhapur. Presently, he is working as Assistant Professor and PG Teacher, Department of Pharmaceutics, Ashokrao Mane College of Pharmacy, Peth Vadgaon. He has 2 years of diploma and 11 years of degree teaching experience. He has published 10 research articles in reputed national and international journals and 4 books on his credit. He has presented various research papers in national and international level conferences and workshops. He is a life member of various professional associations like APTI, MSPC and KPSF.

Mr. Sardar S. Shelake, has completed M.Pharm from Ashokrao Mane College of Pharmacy, Peth Vadgaon in Pharmaceutics. He has qualified GPAT in 2013. Presently he is perusing Ph.D. in Shivaji University, Kolhapur. Currently, he is working as Assistant Professor, Ashokrao Mane Institute of Pharmacy, Ambap. He has published 15 research and 5 review articles in reputed journals and 2 book chapters. He has 1 year at diploma level, 4 years at UG teaching experience. He is affiliated with professional bodies like MSPC.

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SATARA
DIRECTOR
Yashoda Technical Campus
Satara

Mr. Pravin S. Patil, has completed MPharm. from Govt. College of Pharmacy, Karad in Biopharmaceutics. He has qualified GATE in 2003 and 2004. Presently he is perusing PhD. in Shivaji University, Kolhapur. Presently, he is working as Assistant Professor and PG Head, Ashokrao Mane College of Pharmacy, Peth Vadgaon. He has published 10 research and 3 review articles in reputed journals. He has 2 years at diploma level, 11 years at UG and PG teaching experience. He is affiliated with professional bodies like MSPC, APTI and KPSF.

Dr. D. A. Bhagwat, has completed his B. Pharm. in 2006 from BVCP, Kolhapur, M. Pharm. in Industrial Pharmacy from S. N. Institute of Pharmacy, Pusad in 2008 and Ph.D. from JJT, University, Rajasthan in 2014. He has total 11 years of Teaching Experience. Currently he is working as Assistant Professor Dept. of Pharmaceutics, Bharati Vidyapeeth College of Pharmacy, Kolhapur. He has authored Six books and published 42 research papers in national and international journals and presented more than 60 papers in various national and international conferences. He is life member of IPA and APTI. He has delivered many lectures in Patient Counseling Course conducted by MSPC, Mumbai.

Dr. Shitalkumar S. Patil, M. Pharm. PhD., MBA presently working as Principal, Ashokrao Mane College of Pharmacy, Peth Vadgaon. He has total 29 years of teaching experience. He has authored 5 books and published more than 75 research article in national and international journals. He is life member of APTI. He is a member of Board of Studies, Shivaji University, Kolhapur.

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In silico study for the prediction of multiple pharmacological activities of novel hydrazone derivatives

Sachin H Rohane* & Ashlesha G Makwana

Department of Chemistry, K. B. Institute of Pharmaceutical Education and Research, Gandhinagar 382 023, India

E-mail : sachinrohane29@gmail.com

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The present studies are aimed to predict multiple pharmacological activities of novel hydrazone derivatives. Molecular docking of compounds 1 to 51 have been performed in Small-Molecule Drug Discovery Suite of Schrödinger. Fifty one compounds have been targeted on seven enzymes *viz*. 2NSD and 2X22 involved in tuberculosis activity, 4COX and 3LN1 involved in inflammation, 4GCP and 4HL2 involved in bacterial infection and 4WMZ involved in fungal infection. The generated lower energy conformers of all ligands have been docked into generated grid of active site of enzymes by XP precision of docking inside Glide-v7.4. Molecular docking results suggest that the compounds 4, 5, 11, 18, 30, 34, 35, 37, 38, 42, 43, 44, 45, 46 and 47 have good docking score and are predicted to interact with all enzymes. In all fifteen novel hydrazone derivatives have been predicted for multiple pharmacological activities.

Keywords: Hydrazone, molecular docking, multiple pharmacological activities

In hydrazone, the nitrogen is attached to hydrogen; these hydrazone are stable enough for isolation. However, in some cases, especially with simple R group, they rapidly decompose or polymerize unless there is at least one aryl group on nitrogen or the carbon. When there is an aryl group the compound are quite stable and these compound called Schiff bases and the reaction is the best way to prepare them. The reaction is straightforward and proceeds in high yield[1].

Hydrazones with an azomethine −NHN=CH− proton constitute an important class of compounds for new drug development. Therefore, many researchers have synthesized these compounds as target structures and evaluated their biological activities. These observations have been promoting and guiding the development of new hydrazones that possess multiple biological activities. Hopefully, this will allow the development of innovative new strategies for the development of novel compounds with different schemes, methods and materials[2].

The chemistry of these derivatives has been a fascinating field of investigation in medicinal chemistry. They have been found to exhibit enhanced biological profile. Hydrazones are known to exhibit a wide variety of biological activities. They are used as antibacterial agents, anti-tubercular agents, analgesics, anti-inflammatory agents, antiviral agents, antifungal agents, muscle relaxants and antihistamines, *etc*.[3]

The docking is a computational method to study the formation of intermolecular complexes and is a subject of intensive research. Drug exerts its biological activity by binding to the pocket of receptor molecule. In their binding conformations, the molecules exhibit geometric and chemical complementarily, both of which are essential for successful drug activity. The computational process of searching for a ligand that is able to fit both geometrically and energetically into the binding site of a protein is called molecular docking.

Molecular docking helps in studying drug/ ligand or receptor/ protein interactions by identifying the suitable active sites in protein, obtaining the best geometry of ligand - receptor complex and calculating the energy of interaction for different ligands to design more effective ligands. The target or receptor is either experimentally known or theoretically generated through knowledge based protein modeling or homology modeling. The molecular docking tool has been developed to obtain a preferred geometry of interaction of ligand - receptor complexes having minimum interaction energy based on different scoring functions. This utility allows one to screen a set of compounds for lead optimization or synthesis[4,5].

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Experimental Section

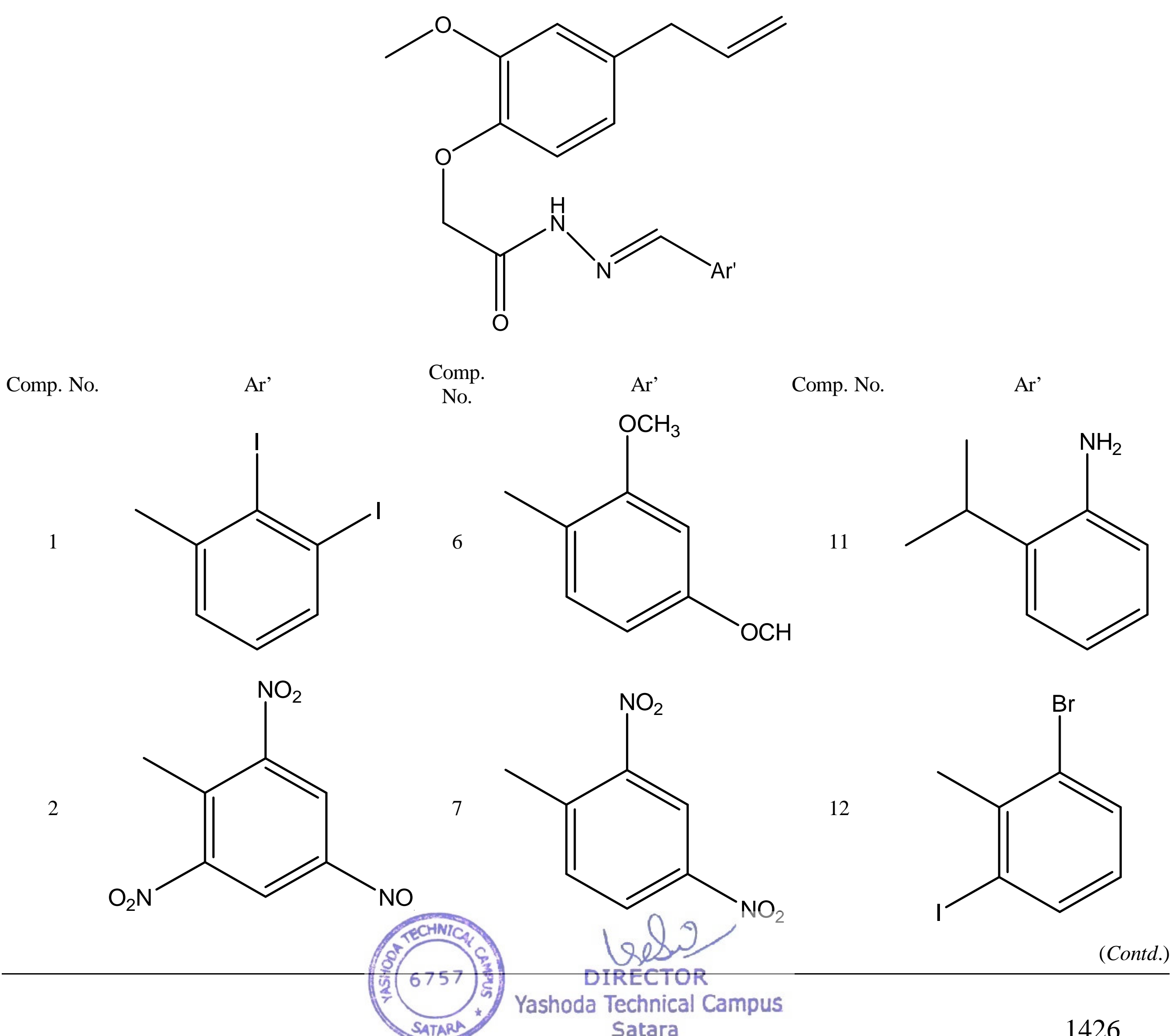
Molecular docking of 1 to 51 compounds was performed in Small-Molecule Drug Discovery Suite of Schrödinger. Fifty one compounds were targeted on 7 enzymes such as 2NSD and 2X22[13] involved in tuberculosis activity, 4COX[8,9] and 3LN1[6,7,10] involved in inflammation[11,12], 4GCP and 4HL2 involved in bacterial infection and 4WMZ involved in fungal infection. The generated lower energy conformers of all ligands were docked into generated grid of active site of enzymes by XP precision of docking inside Glide-v7.4.

Preparation of small molecule: A set of new 51 hydrazone derivatives (Table I) were compiled by us using ChemDraw. 3D structures which were constructed using Chem 3D ultra 12.0 software [Molecular Modeling and Analysis; Cambridge Soft Corporation, USA (2010)], and saved as MDL Mol File (* .mol).

Ligand preparation

The structure of each compound was cleaned and optimized using Ligprep. The clean-up and optimization process include conversion of structures from 2D to 3D, addition of hydrogen atoms, generation of possible ionization state at *p*H 7.0, generation of tautomers (if any), generation of all combinations of stereoisomers, and energy minimization. The low energy conformer of ligands was generated using OPLS3 force field. All structures were saved in 'maestro' output format.

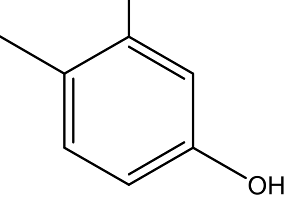
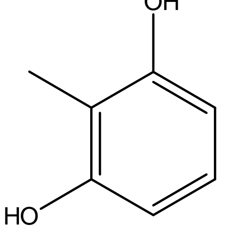
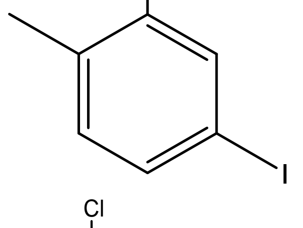
Table I — List of compounds



(*Contd.*)

Table I — List of compounds (*Contd.*)

O O H N N Ar' O

Comp. No.	Ar'	Comp. No.	Ar'	Comp. No.	Ar'
3	Cl, Cl	8	Cl, Cl	13	Br, I
4		9		14	Br, I
5		10	NO_2, O_2N	15	Cl
16	Cl, N	21	OH, I, Cl	26	I, I

(*Contd.*)

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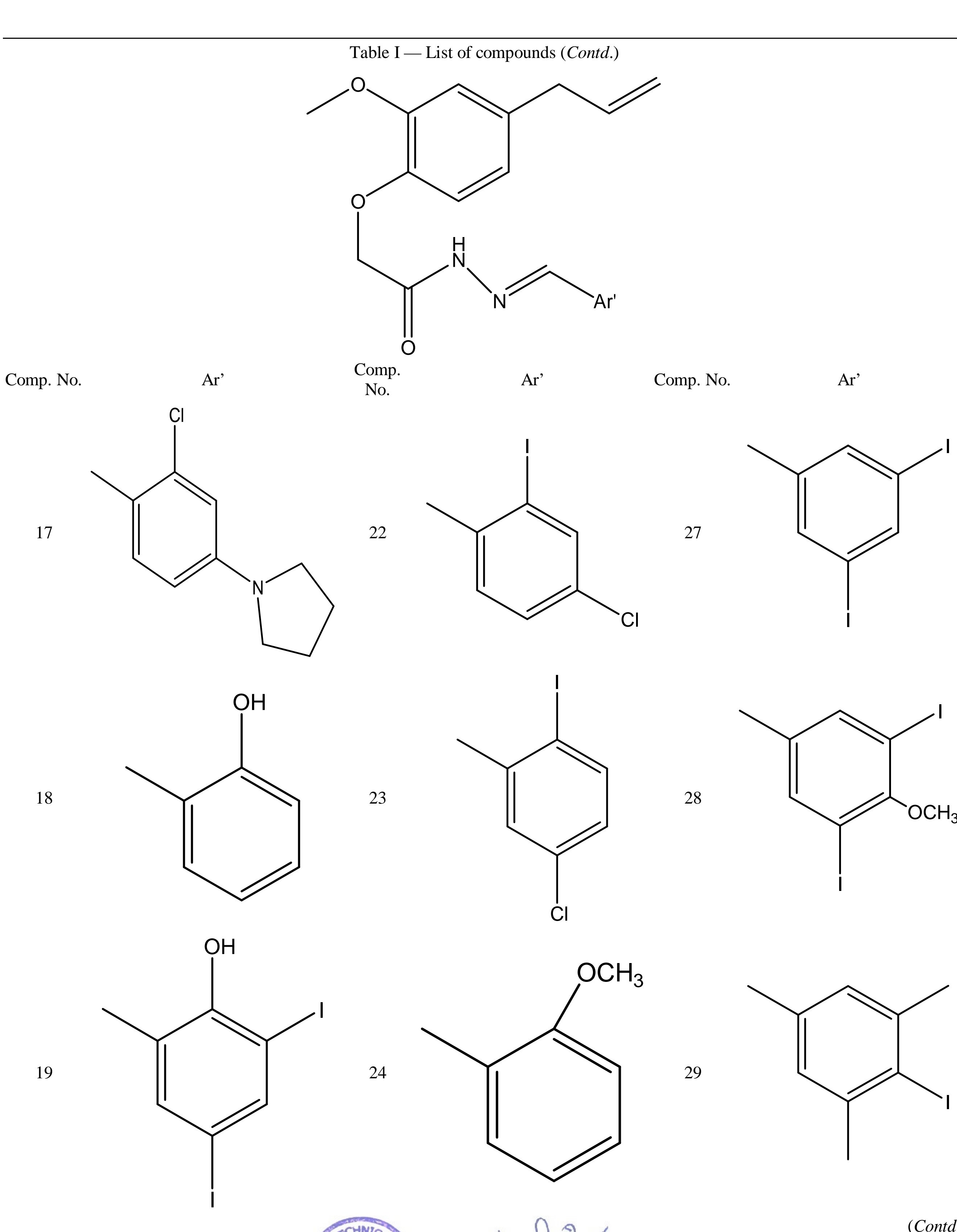
Table I — List of compounds (*Contd.*)



(*Contd.*)

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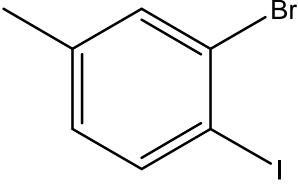
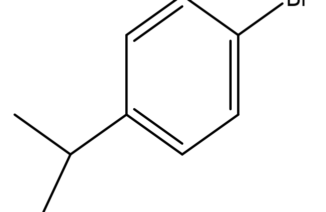
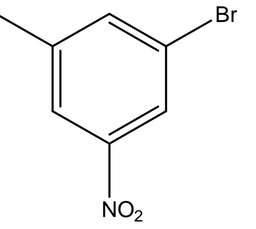
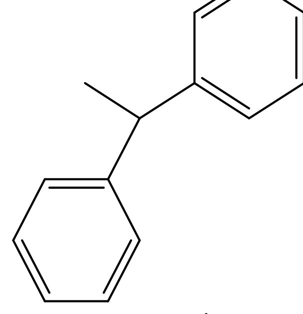
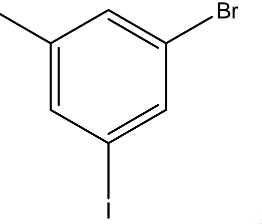
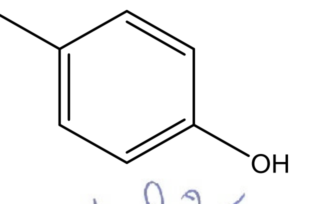
Table I — List of compounds (*Contd.*)

Comp. No.	Ar'	Comp. No.	Ar'	Comp. No.	Ar'
20		25		30	
31		38		45	
32		39		46	
33		40		47	

(*Contd.*)

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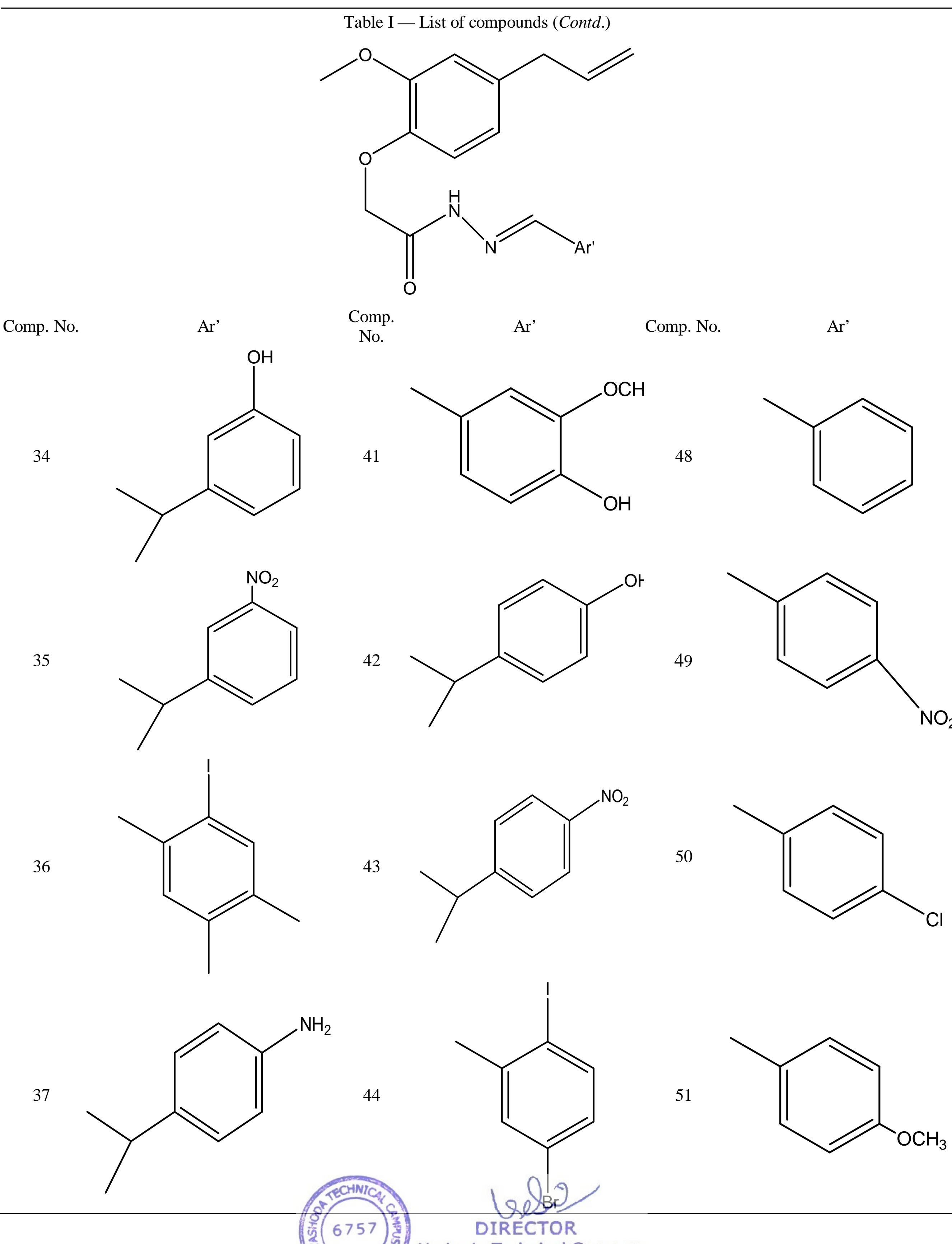
Table I — List of compounds (*Contd.*)



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Protein preparation

The protein such as 2NSD, 2X22, 4COX, 3LN1, 4GCP, 4HL2 and 4WMZ download from protein data bank. All protein structures were prepared for docking using the ‘‘protein preparation wizard’’ in Maestro-v11.1. The protein preparation was carried out in two steps, preparation and refinement. Preparation steps involved assigning of bond order, addition hydrogen, removal of original hydrogen, creation of zero order bonds to metals, and filling of missing side chain an d loops using prime. All water molecules in the crystal structures were deleted, het states of bound ligands were generated using Epik at *p*H 7.0 and termini were capped by adding ACE and NMA residue. The refinement steps involved the optimization of hydrogen bonding network was by reorienting hydroxyl groups, and amide groups of Asn and Gln, and selecting appropriate states and orientations of the imidazole ring in His residues. The refinement steps also include a restrained impact minimization of the co-crystallized complex. It uses the OPLS3 force field for this purpose.

Grid Generation

Grid files represent the active sites of enzyme that are searched when attempting to dock a ligand. It was generated by Receptor Grid Generation panel of Glide-v7.4. Grids were defined by centering on the co-crystallized ligand in the crystal structure. It excludes co-crystallized ligand and thus determines the position and size of the active site. The size of grid box was fixed so that ligand with size of </= 20 Å can be docked. The van der Waals radius scaling factor of 0.7 for atoms with a partial atomic charge (absolute value) less than 0.25 was used to soften the potential for nonpolar parts of the receptor. The constraints were also defined as per various interactions visualized in PDB of co-crystallized ligands with respective enzyme. The rotatable groups like hydroxyl and thiol groups in enzymes were allowed to rotate.

Docking calculations

The generated lower energy conformers of all ligands were docked into generated grid of active site of enzymes by XP precision of docking inside Glide-v7.4. The ligand sampling was set up to be flexible which allow the sampling of ring conformation, nitrogen inversion and penalize the non planar conformation of amide groups. Some more settings were set like adding of epic state penalties to docking score, rewarding intramolecular hydrogen bonds, and enhancement of planarity of conjugated pi-groups. The constraints were selected as set in grid generation, must be satisfied for at least 1 interaction. All other advanced settings were set as defaults given in software.

Results and Discussion

The docking study was performed using Small-Molecule Drug Discovery Suite of Schrödinger Glide-v7.4. All novel hydrazone derivatives were docked into the active site of seven enzymes such as enoyl acyl carrier protein reductase inhA (PDB ID: 2NSD), *M. tuberculosis* inhA (PDB ID: 2X22), COX-2 (PDB ID: 4COX), COX-2 (PDB ID: 3LN1), *E. coli* OmpF porin (PDB ID: 4GCP), New delhi metallo-beta-lactamase-11.05 A (PDB ID: 4HL2) and *S. cerevisiae* CYP51 (PDB ID: 4WMZ) which showed better docking scores than the reference compounds (Table II). There are fifteen compounds which showed a fit interaction with enzymes (Figures 1-14) and predicted for multiple pharmacological activities (Table III).

Table II — Docking score of compounds

Compounds	Enoyl acyl carrier protein reductase inhA PDB ID: 2NSD	M. tuberculosis inhA PDB ID:2X22	COX-2 PDB ID:4COX	COX-2 PDB ID:3LN1	E.coli OmpF porin PDB ID: 4GCP	NDM beta-lactamase PDB ID:4HL2	S. cerevisiae CYP51 PDB ID: 4WMZ
Isoniazide	−3.682	−4.682	–	–	–	–	–
Indomethacin	–	–	−10.739	−6.157	–	–	–
Ampicillin	–	–	–	–	−5.547	−11.363	–
Fluconazole	–	–	–	–	–	–	−4.709
1	−8.747	−5.959	−8.383	–	–	−4.247	−4.732
2	−6.847	−4.009	–	–	−2.959	−4.665	−4.572
3	–	−8.007	−8.257	–	−1.63	−5.972	−6.508

(*Contd.*)

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Table II — Docking score of compounds (*Contd.*)

Compounds	Enoyl acyl carrier protein reductase inhA PDB ID: 2NSD	M. tuberculosis inhA PDB ID:2X22	COX-2 PDB ID:4COX	COX-2 PDB ID:3LN1	E.coli OmpF porin PDB ID: 4GCP	NDM beta-lactamase PDB ID:4HL2	S. cerevisiae CYP51 PDB ID: 4WMZ
4	−10.393	−8.426	−9.265	−7.791	−4.511	−6.898	−8.428
5	−7.919	−6.09	−8.442	−6.428	−1.5	−4.423	−6.679
6	−7.247	−	−7.668	−	−2.714	−5.027	−
7	−7.179	−6.67	−5.952	−	−	−5.166	−6.291
8	−8.387	−5.981	−8.302	−	−2.187	−5.034	−
9	−8.525	−7.538	−7.875	−1.693	−3.142	−5.923	−6.864
10	−7.099	−	−6.187	−	−2.405	−4.581	−5.751
11	−9.5	−7.726	−8.955	−	−3.181	−5.374	−8.413
12	−6.942	−4.965	−	−	−	−4.264	−6.665
13	−8.921	−	−7.069	−	−	−4.427	−
14	−8.216	−8.073	−6.788	−	−	−4.68	−6.013
15	−7.428	−6.721	−7.81	−	−	−5.311	−4.785
16	−7.815	−2.124	−8.288	−	−	−4.998	−
17	−7.266	−6.04	−8.619	−4.222	−	−4.124	−6.796
18	−7.632	−6.625	−9.066	−	−2.803	−7.064	−6.546
19	−7.681	−5.954	−8.421	−4.79	−1.941	−6.277	−4.722
20	−7.466	−4.423	−8.086	−	−2.969	−7.064	−6.602
21	−6.799	−7.037	−8.669	−6.662	−3.806	−5.954	−7.407
22	−7.005	−5.353	−8.992	−	−1.627	−4.757	−6.903
23	−7.867	−	−8.302	−	−3.138	−5.497	−6.266
24	−7.883	−4.742	−7.439	−6.857	−3.146	−5.18	−
25	−8.657	−6.098	−	−	−1.774	−5.192	−6.006
26	−8.15	−4.74	−	−	−	−5.294	−7.637
27	−7.37	−	−8.443	−5.13	−	−4.478	−5.638
28	−8.101	−7.486	−	−	−2.672	−4.591	−
29	−6.097	−2.37	−8.057	−	−2.145	−4.536	−4
30	−9.021	−7.769	−8.387	−5.29	−4.511	−6.344	−7.069
31	−7.804	−6.092	−8.034	−	−	−4.533	−5.082
32	−6.384	−4.919	−7.632	−4.498	−	−3.956	−5.302
33	−6.955	−6.071	−6.671	−	−3.023	−4.951	−5.997
34	−10.13	−7.448	−8.813	−6.432	−	−5.596	−7.837
35	−9.813	−7.338	−7.143	−5.75	−3.408	−4.739	−5.981
36	−6.538	−6.226	−7.645	−4.353	−2.342	−3.919	−
37	−9.632	−9.092	−9.093	−	−3.923	−5.879	−7.368
38	−9.587	−7.932	−8.51	−5.06	−2.745	−4.878	−6.968
39	−6.818	−5.289	−7.305	−3.918	−3.266	−4.89	−5.633
40	−6.921	−7.674	−7.827	−	−3.537	−5.813	−
41	−6.98	−	−	−7.744	−	−5.297	−6.793
42	−9.747	−8.527	−9.213	−6.714	−4.075	−6.698	−8.273
43	−9.256	−6.551	−8.132	−7.166	−3.418	−5.93	−6.468
44	−8.898	−5.356	−7.439	−	−3.139	−5.436	−6.403
45	−9.049	−6.262	−9.028	−	−3.349	−5.752	−6.648
46	−9.593	−7.758	−8.239	−5.798	−3.107	−5.097	−7.65
47	−9.485	−7.556	−8.945	−5.585	−2.442	−6.18	−7.724
48	−6.469	−4.896	−7.119	−	−1.847	−5.637	−5.377
49	−8.072	−5.725	−7.728	−	−3.171	−5.141	−
50	−6.338	−6.666	−8.549	−	−	−5.029	−5.944
51	−6.175	−4.293	−8.001	−	−1.611	−4.306	−7.02

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Table III — Docking score of compounds with multiple pharmacological activities

Compounds	Enoyl acyl carrier protein reductase inhA PDB ID: 2NSD	M. tuberculosis inhA PDB ID: 2X22	COX-2 PDB ID: 4COX	COX-2 PDB ID: 3LN1	E.coli OmpF porin PDB ID: 4GCP	NDM beta-lactamase PDB ID: 4HL2	S. cerevisiae CYP51 PDB ID: 4WMZ
Isoniazide	−3.682	−4.682					
Indomethacin			−10.739	−6.157			
Ampicillin					−5.547	−11.363	
Fluconazole							−4.709
4	−10.393	−8.426	−9.265	−7.791	−4.511	−6.898	−8.428
34	−10.13	−7.448	−8.813	−6.432	−5.78	−5.596	−7.837
42	−9.747	−8.527	−9.213	−6.714	−4.075	−6.698	−8.273
37	−9.632	−9.092	−9.093	−	−3.923	−5.879	−7.368
45	−9.049	−6.262	−9.028	−	−3.349	−5.752	−6.648
46	−9.593	−7.758	−8.239	−5.798	−3.107	−5.097	−7.65
38	−9.587	−7.932	−8.51	−5.06	−2.745	−4.878	−6.968
30	−9.021	−7.769	−8.387	−5.29	−4.511	−6.344	−7.069
11	−9.5	−7.726	−8.955	−	−3.181	−5.374	−8.413
18	−7.632	−6.625	−9.066	−	−2.803	−7.064	−6.546
47	−9.485	−7.556	−8.945	−5.585	−2.442	−6.18	−7.724
43	−9.256	−6.551	−8.132	−7.166	−3.418	−5.93	−6.468
44	−8.898	−5.356	−7.439	−	−3.139	−5.436	−6.403
5	−7.919	−6.09	−8.442	−6.428	−1.5	−4.423	−6.679
35	−9.813	−7.338	−7.143	−5.75	−3.408	−4.739	−5.981

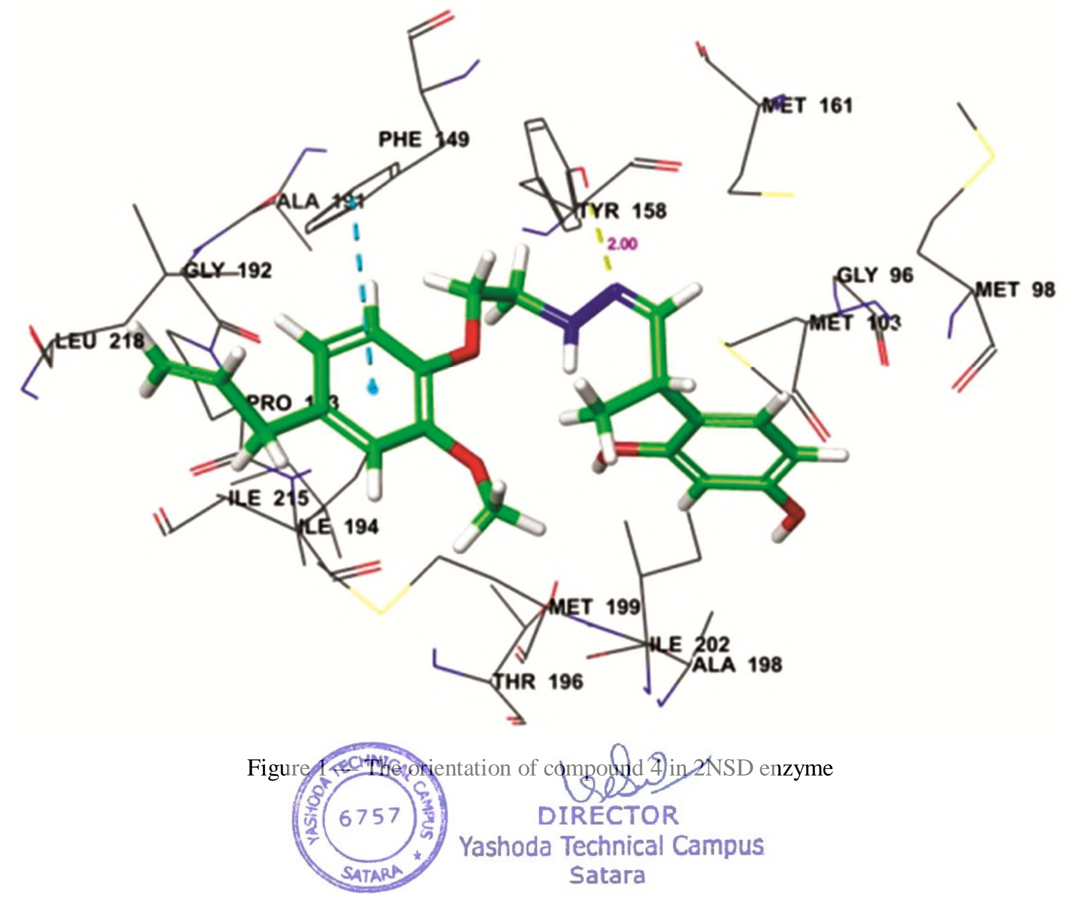

Figure 1 — The orientation of compound 4 in 2NSD enzyme

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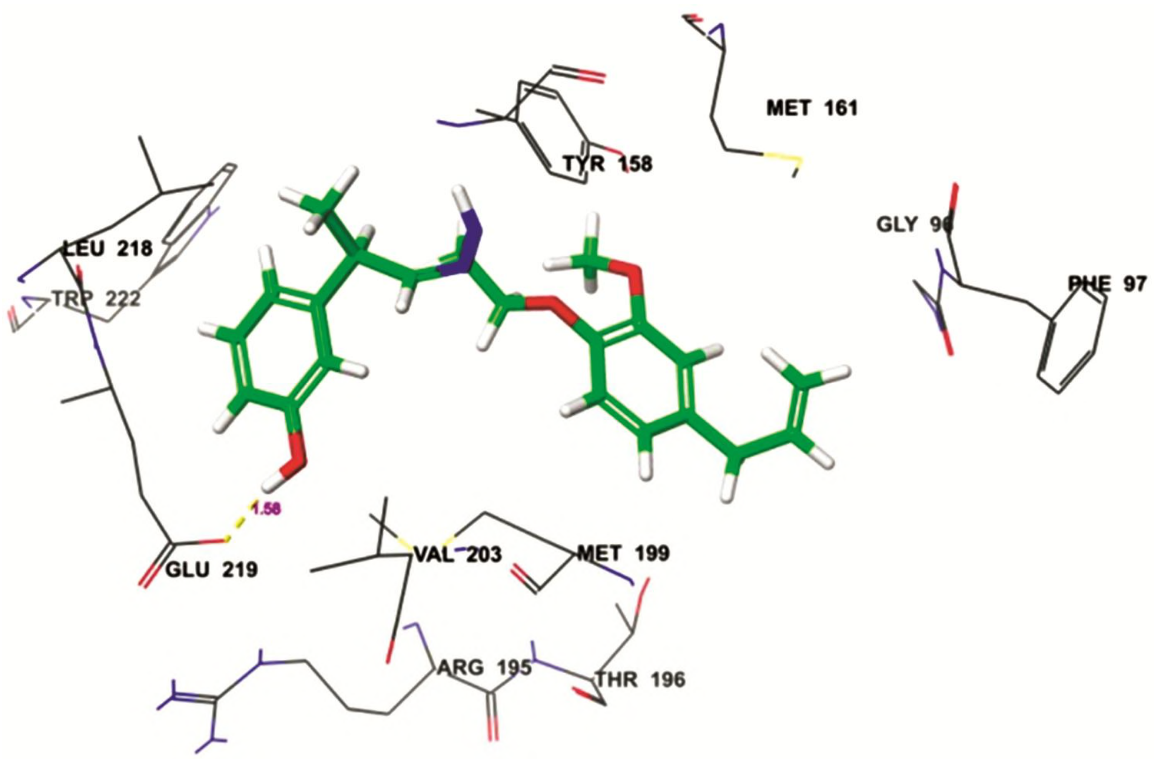

Figure 2 — The orientation of compound 34 in 2NSD enzyme

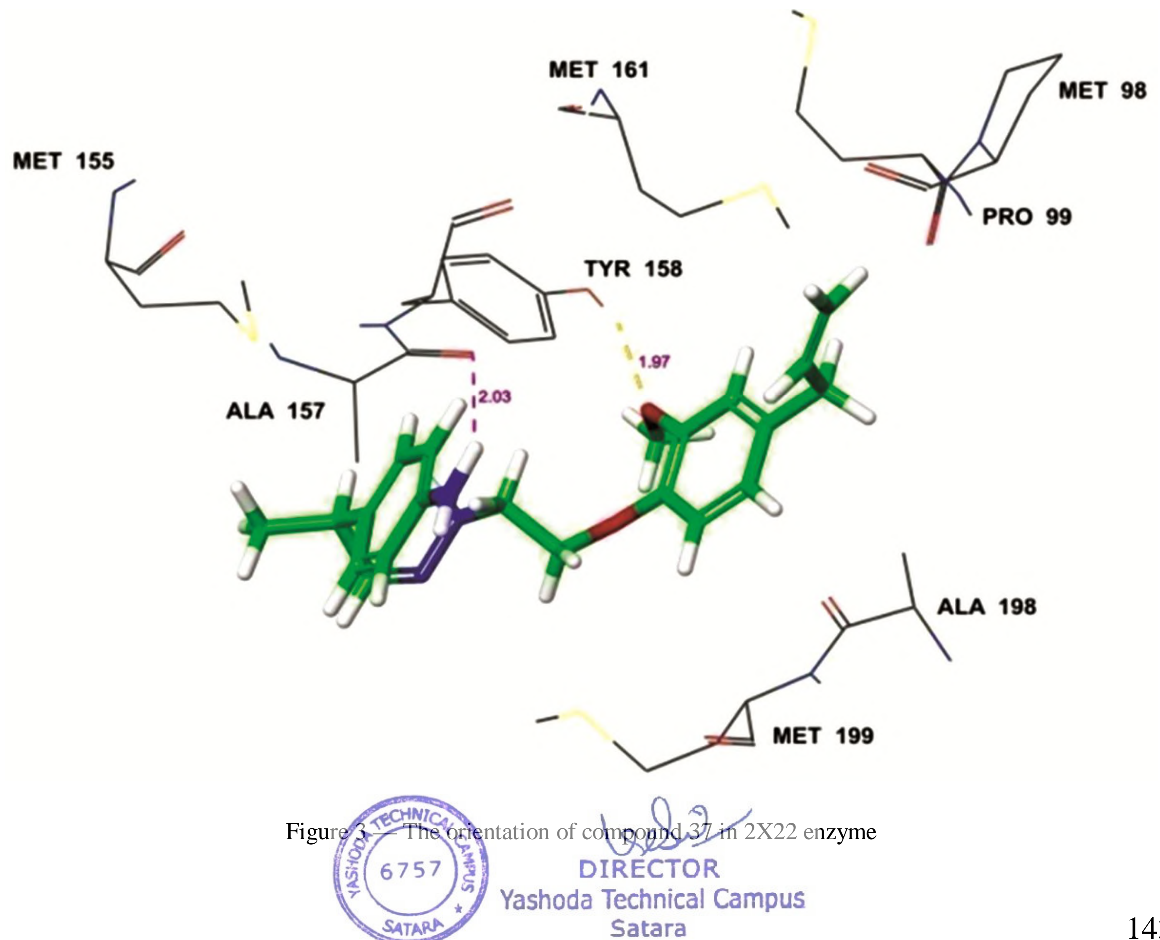

Figure 3 — The orientation of compound 37 in 2X22 enzyme

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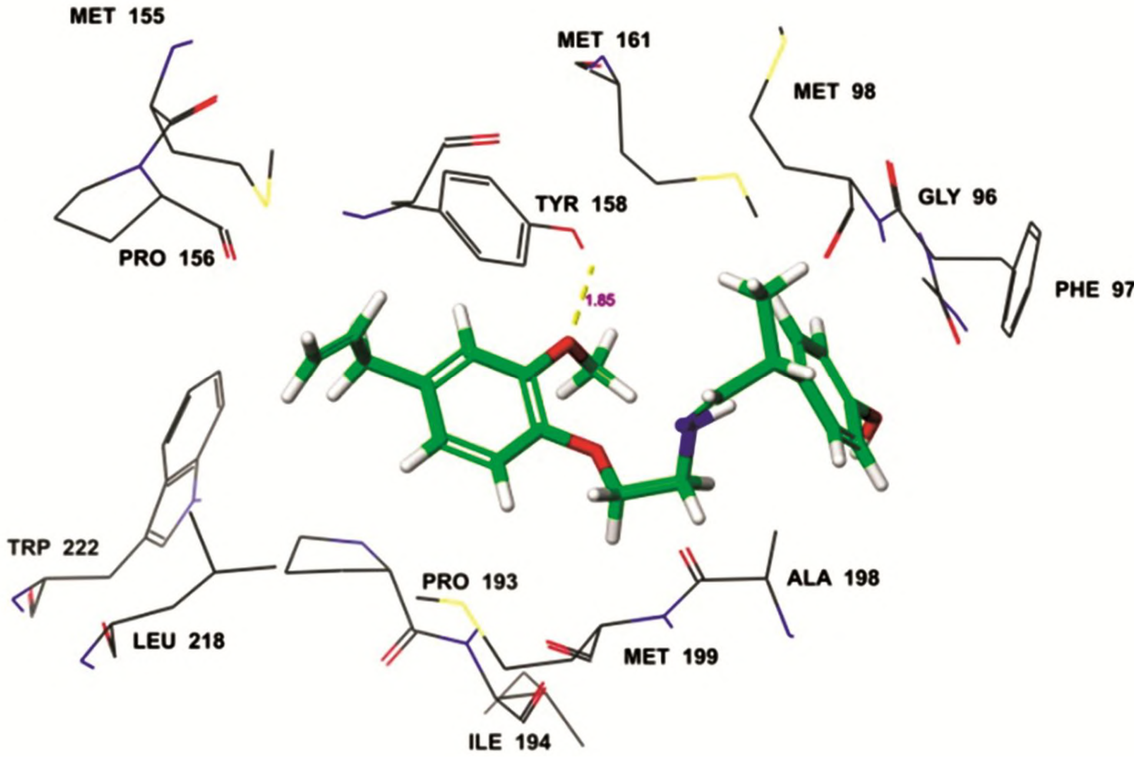

Figure 4 — The orientation of compound 42 in 2X22 enzyme

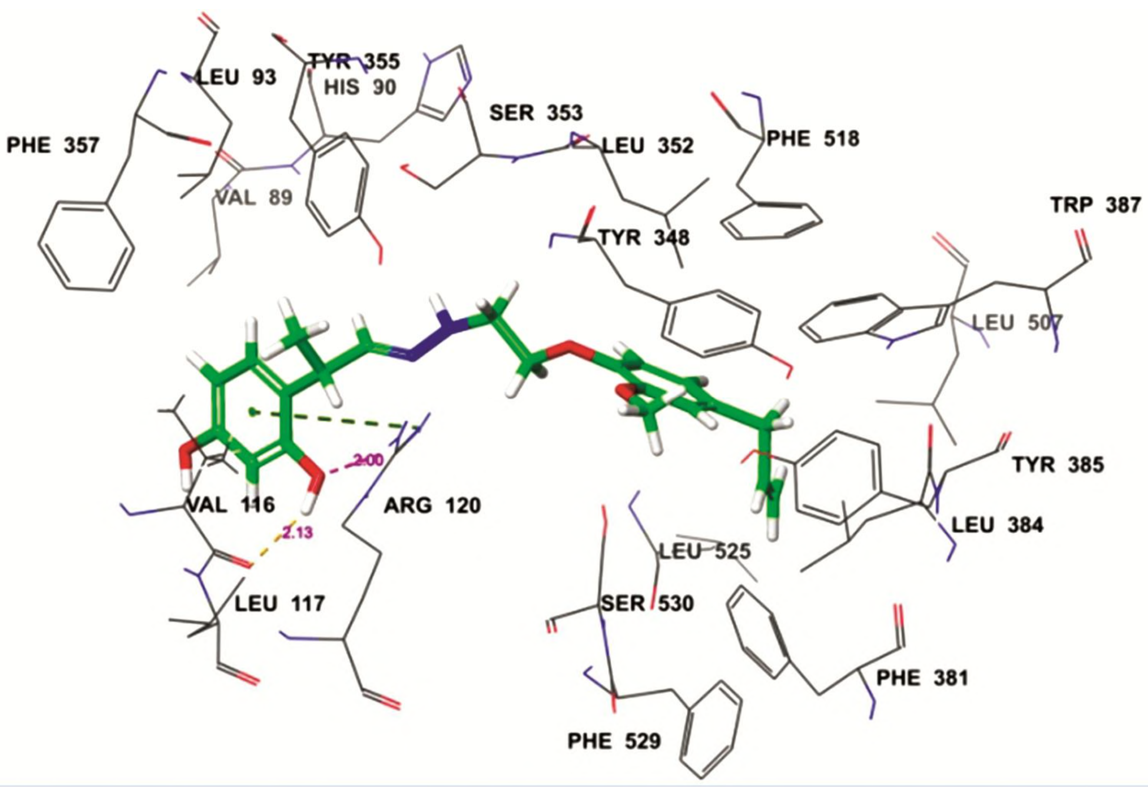

Figure 5 — The orientation of compound 4 in 4COX enzyme

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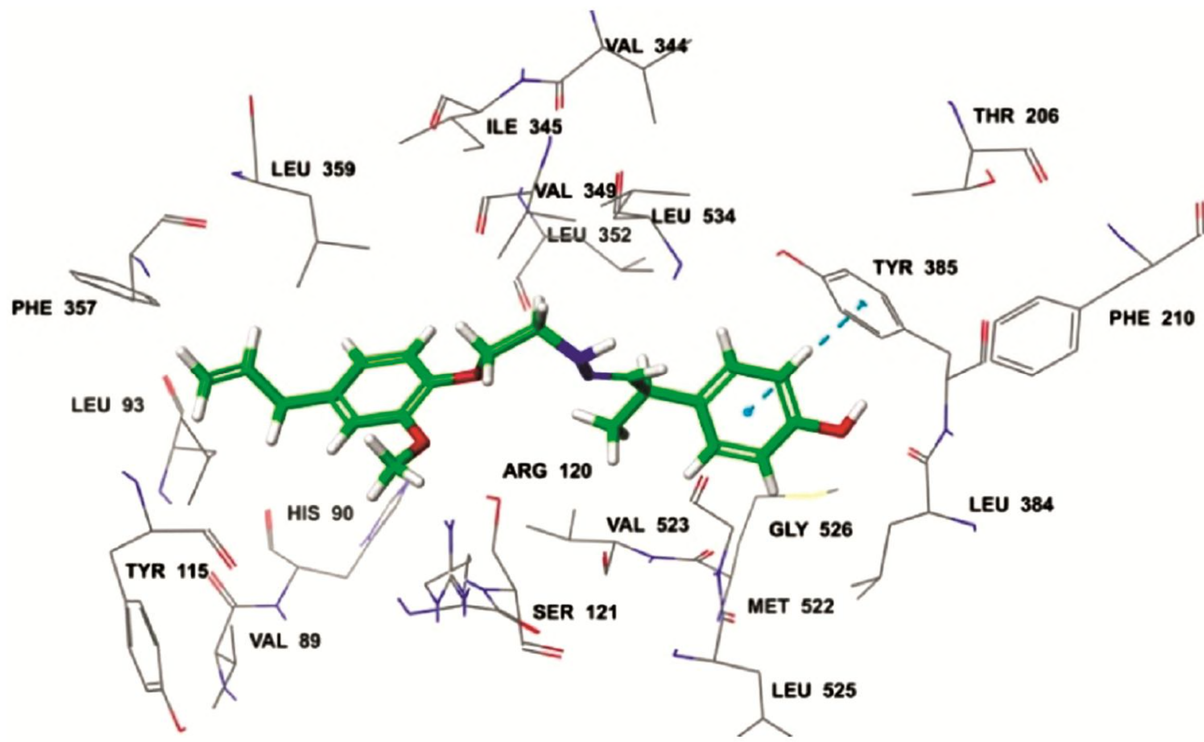

Figure 6 — The orientation of compound 42 in 4COX enzyme

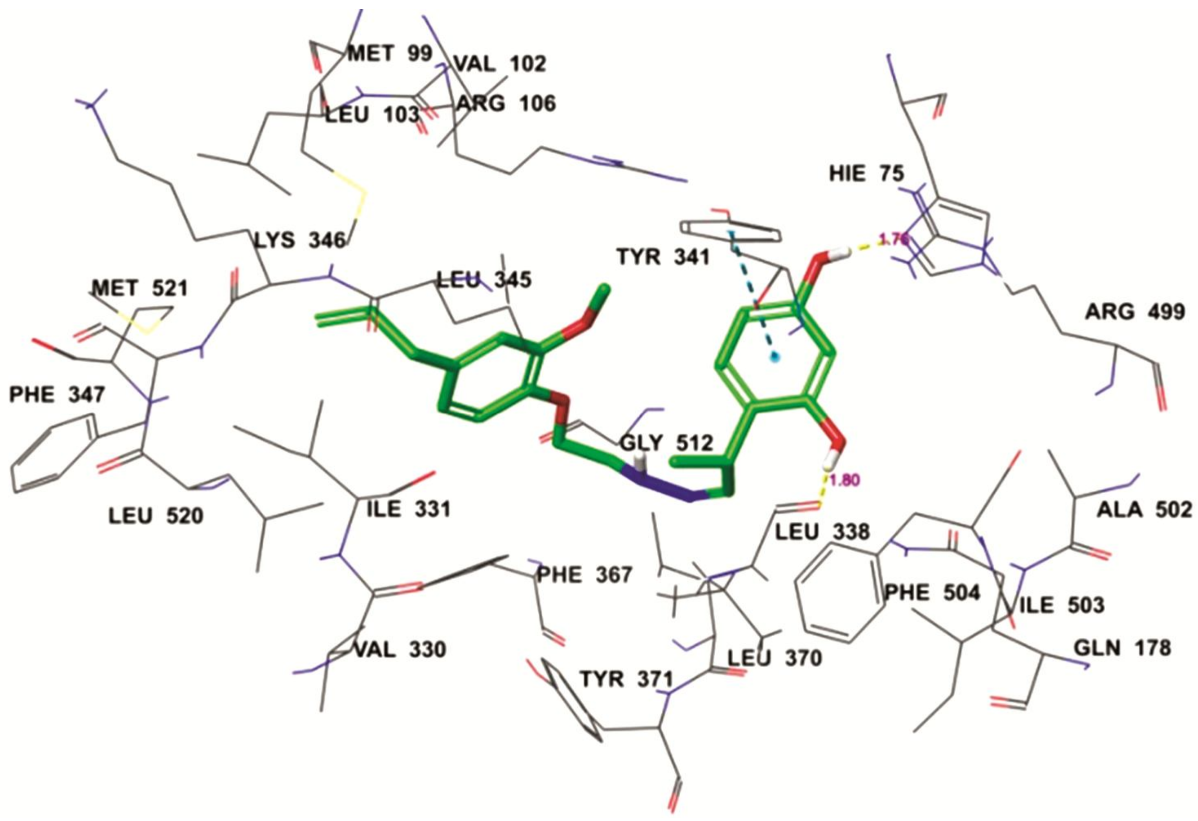

Figure 7 — The orientation of compound 4 in 3LN1 enzyme

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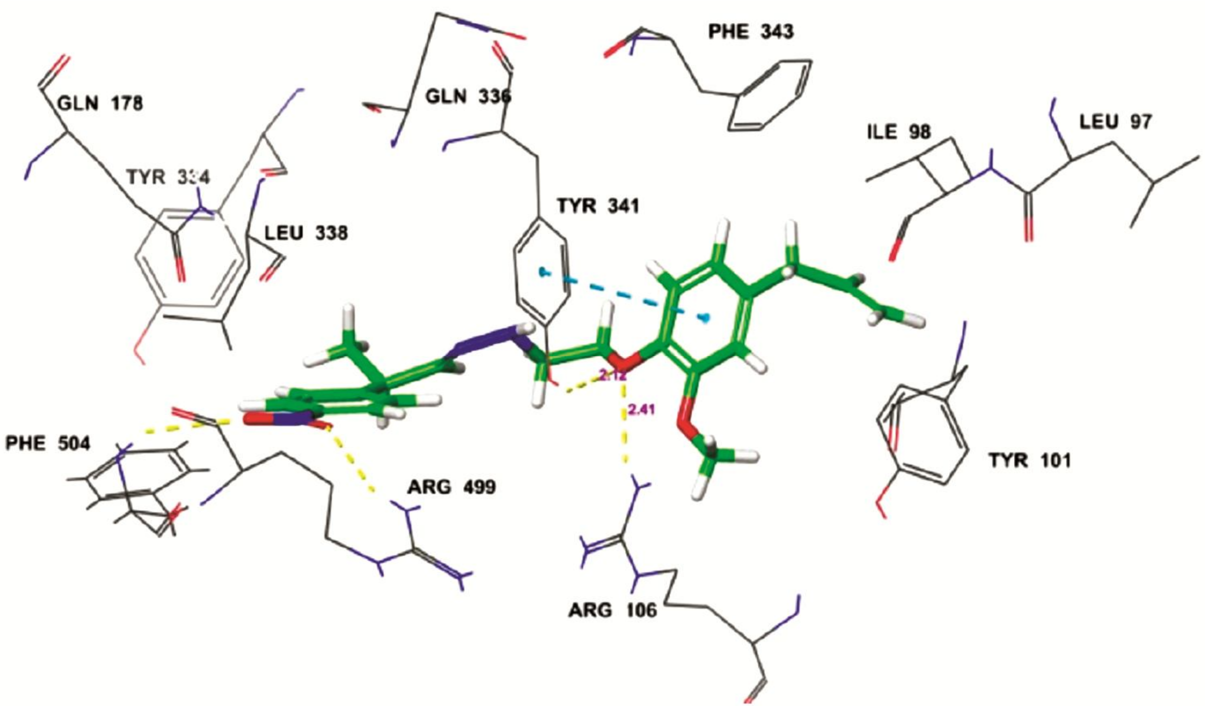

Figure 8 — The orientation of compound 43 in 3LN1 enzyme

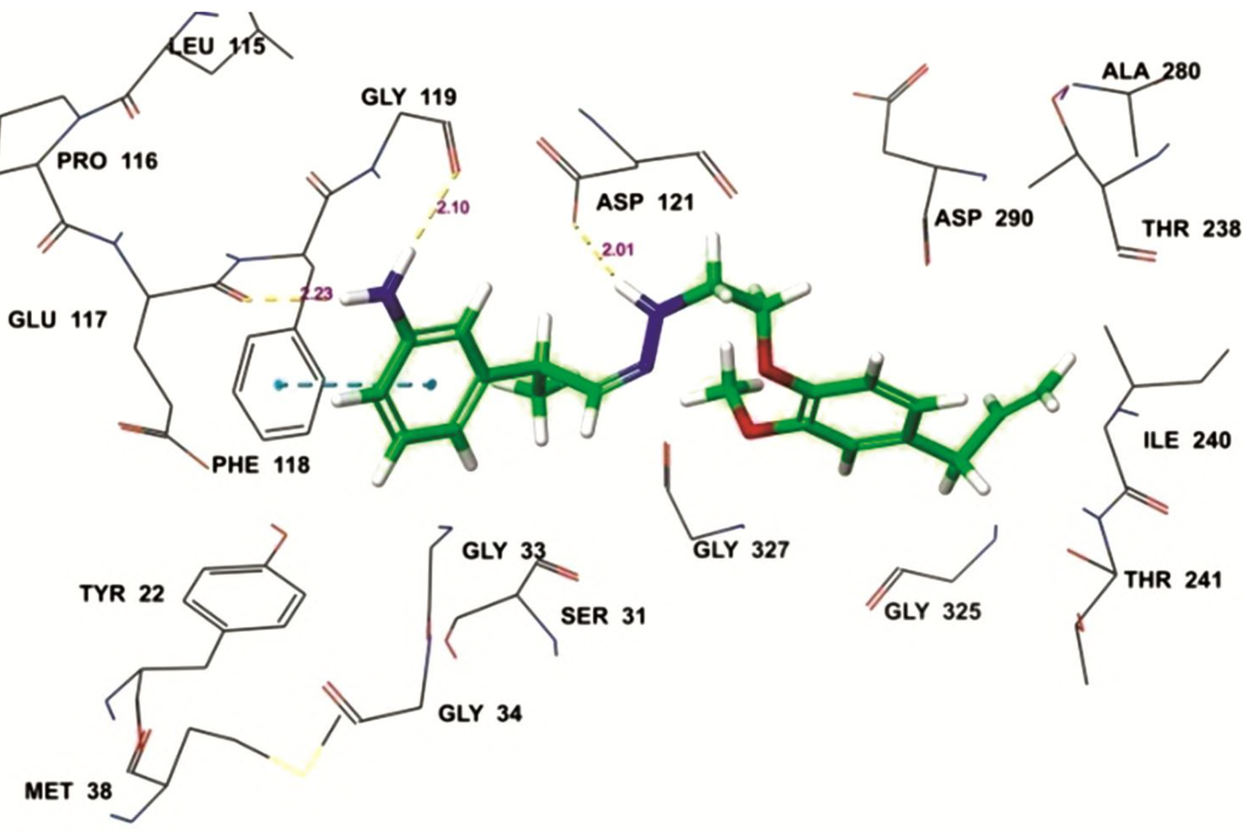

Figure 9 — The orientation of compound 30 in 4GCP enzyme

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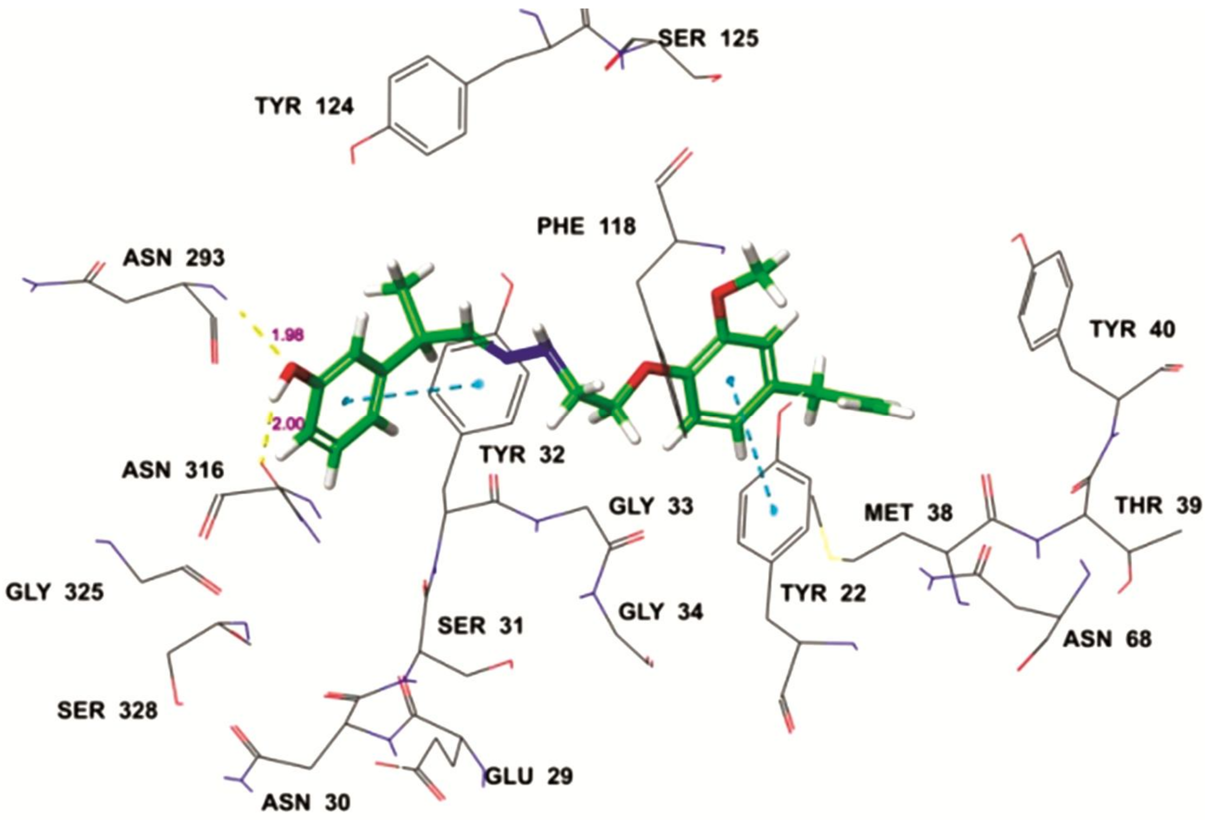

Figure 10 — The orientation of compound 34 in 4GCP enzyme

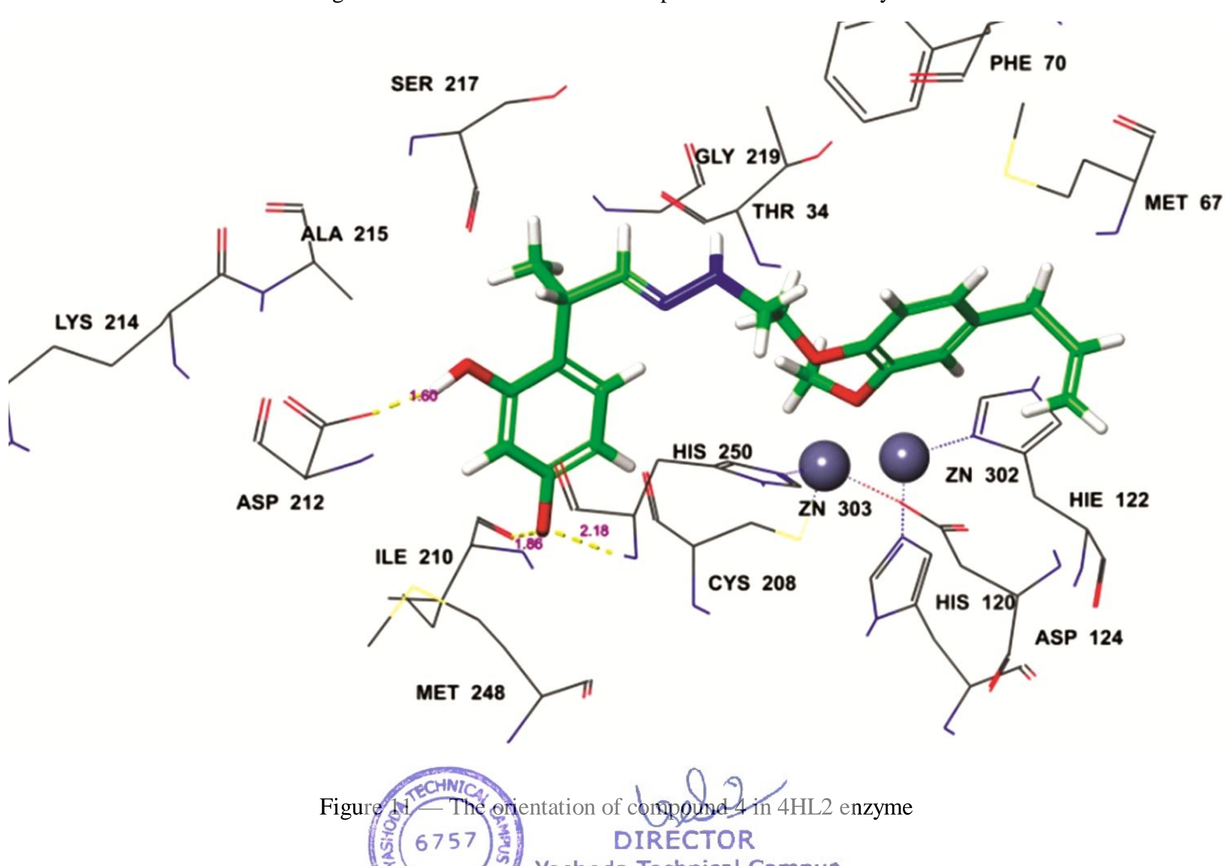

Figure 11 — The orientation of compound 4 in 4HL2 enzyme

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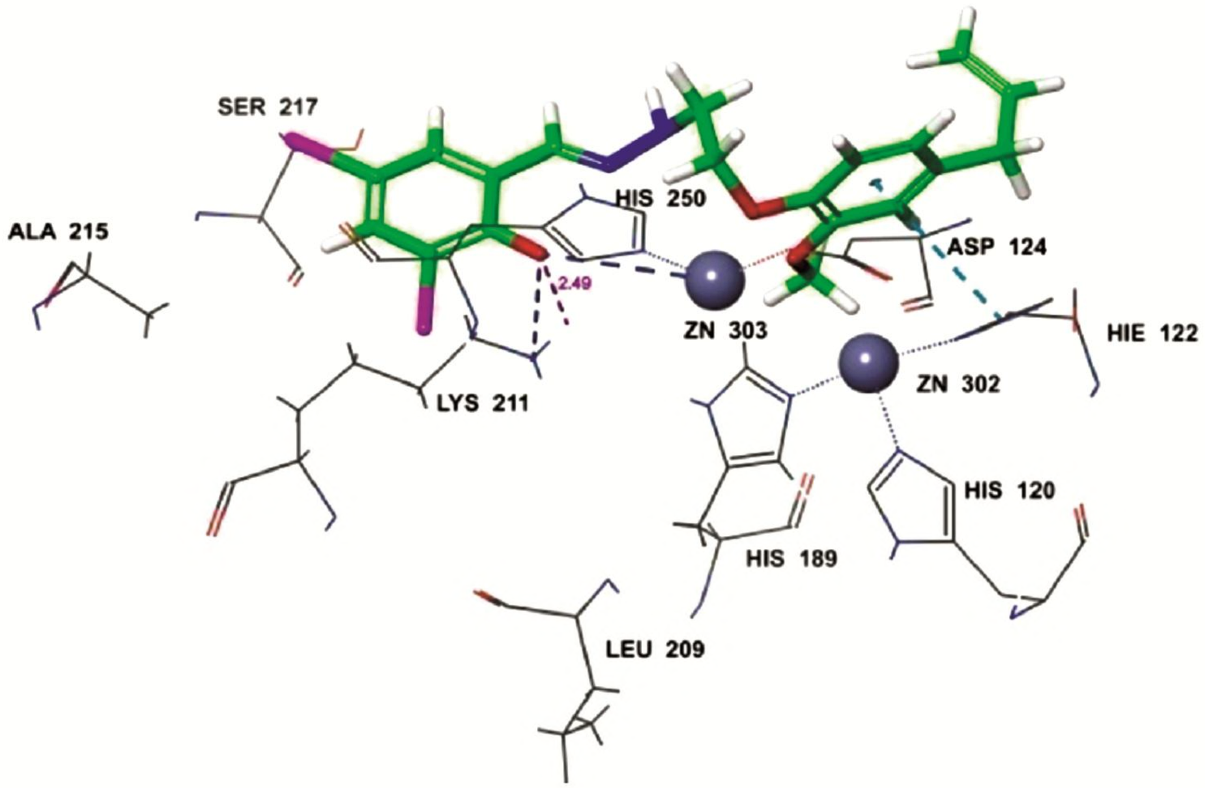

Figure 12 — The orientation of compound 18 in 4HL2 enzyme

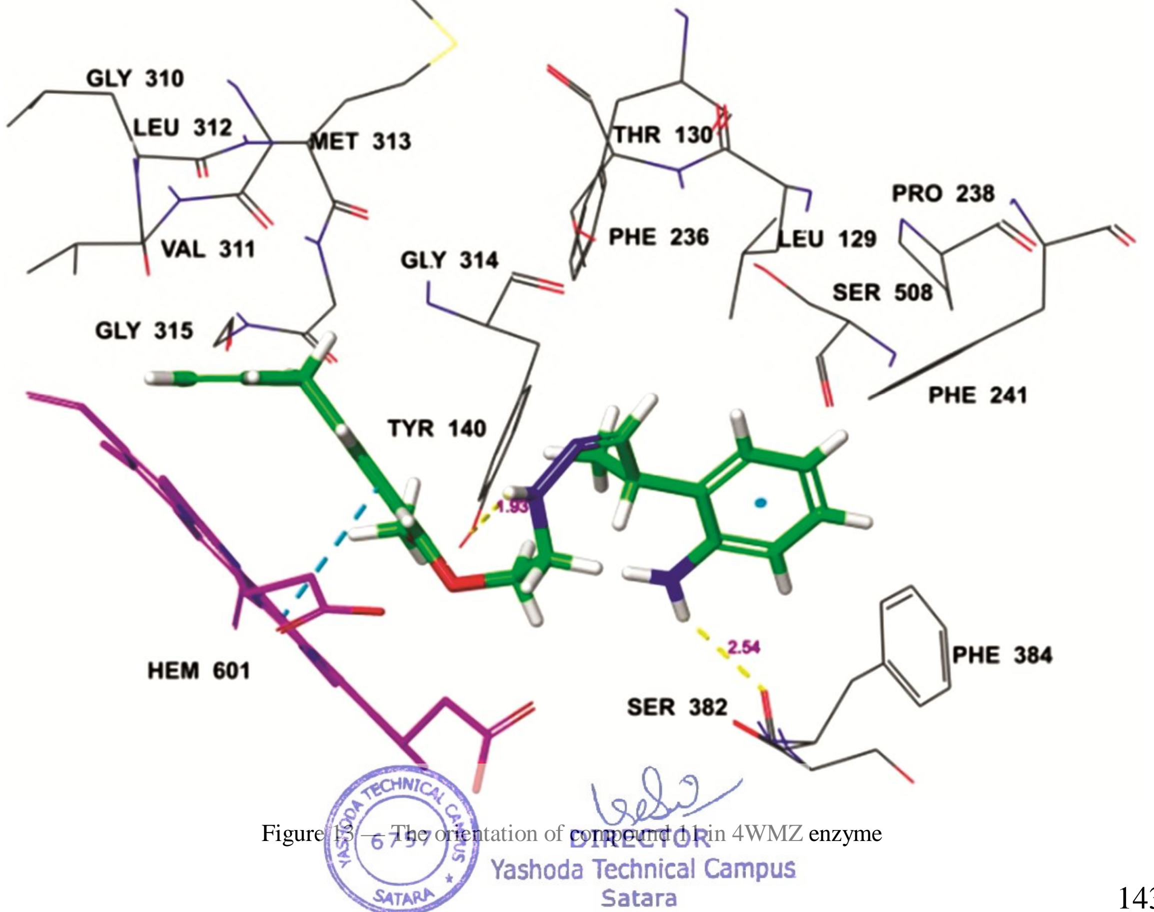

Figure 13 — The orientation of compound 11 in 4WMZ enzyme

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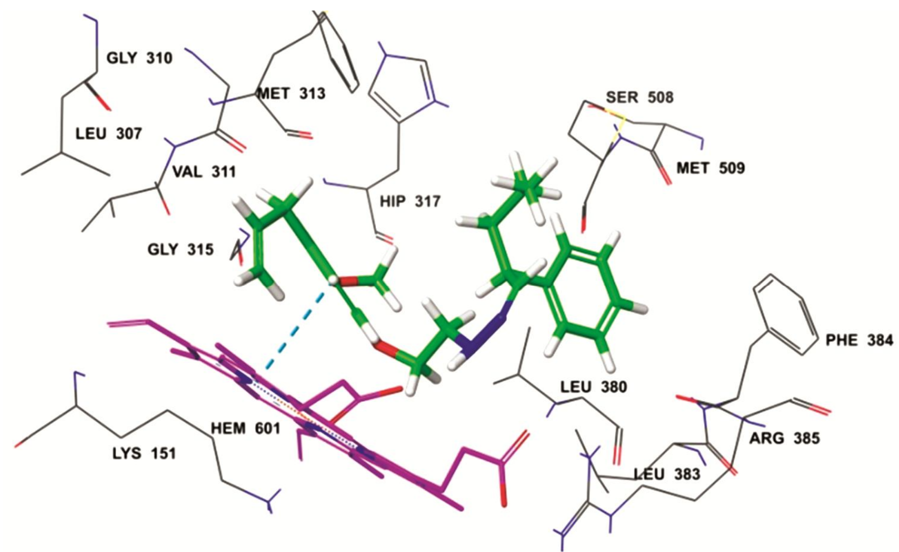

Figure 14 — The orientation of compound 47 in 4WMZ enzyme

Conclusion

Molecular docking has gaining consideration as an important tool for drug discovery. The molecular docking studies help in understanding the various interactions between the ligands and enzyme active sites in detail and thereby help to design novel hydrazones. The docking experiments were carried out for all the fiftyone compounds on seven enzymes and compared the docking score with reference compounds. The compounds 4, 5, 11, 18, 30, 34, 35, 37, 38, 42, 43, 44, 45, 46 and 47 showed higher binding score. These compounds are predicted for multiple pharmacological activities such as antitubercular, anti-inflammatory, antibacterial and antifungal.

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RESEARCH ARTICLE

Extraction, Characterization and Functionalization of Tamarind Gum

Kailas K. Mali[1]*, Shashikant C. Dhawale[2], Remeth J. Dias[3]
[1]Department of Pharmaceutics, YSPM'S Yashoda Technical Campus, Faculty of Pharmacy, Wadhephata, Satara 415011, Maharashtra, India.
[2]Department of Pharmacology, School of Pharmacy, SRTM University, Nanded, 431606, Maharashtra, India
[3]Department of Pharmacy, Government Polytechnic, National Highway No 6, Jalgaon 425002, Maharashtra, India
*Corresponding Author E-mail: **malikailas@gmail.com**

ABSTRACT:
The aim of present work was to extract, modify and characterize tamarind gum (TG), and explore its pharmaceutical applications. TG was extracted from tamarind kernel powder and tamarind seeds, and modified to carboxymethyl tamarind gum (CMTG) by using monochloroacetic acid. TG and CMTG were evaluated for pH, solubility, viscosity, swelling and powder characteristics, and characterized by Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy, solid state ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectroscopy, differential scanning calorimeter (DSC) and X-ray diffractometer. TG was successfully extracted with yield more than 50%. Extracted gum was free from impurities like proteins and fats. Carboxymethylation of TG was confirmed by ATR-FTIR, solid state ^{13}C-NMR and DSC study. The result of XRD study indicates that the amorphous nature of CMTG. Functionalization of TG into carboxymethyl derivative improved physicochemical properties of TG. CMTG showed high solubility, viscosity and swelling than TG. Results of the study revealed that carboxymethyl derivative can be potentially used for development of various drug delivery systems. It can be concluded that the CMTG has great potential as an excipient in pharmaceutical industry.

KEYWORDS: Extraction, Characterization, Tamarind gum, Carboxymethyl tamarind gum, Functionalization of tamarind gum.

1. INTRODUCTION:
The attention towards polysaccharides of natural origin is constantly rising during the past decade. The natural polysaccharides are widely used in the field of food technology, cosmetics, pharmaceuticals and biomedical sciences. Exploitation of new sources of polysaccharides of different origin is well documented in the literature[1]. They exhibit good mechanical properties and are widely used as fibers, films, adhesives, rheology modifiers, hydrogels, emulsifiers and drug delivery agents. Sodium alginate (SA), xanthan gum (XG), guar gum, scleroglucan, and locust bean gums are some of the natural polysaccharides which are fueling the interest of the researchers dealing with the development of drug delivery systems[2].

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The functional groups of polysaccharides have been explored for chemical modification to change their properties like solubility, swelling, viscosity, and degradation[3].

Tamarind seed contains approximately 65% of the gum and it may be used for the development of specific drug delivery systems. The polysaccharide that is present in tamarind gum (TG) is known as tamarind seed polysaccharide[4]. Functionalization of gum into Carboxymethyl tamarind gum (CMTG) may improve physicochemical properties of tamarind gum. Despite being well suited for pharmaceutical application, TG exhibits some potential drawbacks. TG has a dull color and unpleasant odor. Its insolubility in water and degradation in aqueous environment has forced the scientists to chemically modify its functional groups[5]. Various modifications which have been executed till date include carboxymethylation[6], acetylation[7], hydroxyl-alkylation[8] and thiolisation[9,10]. Such

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modifications have caused alteration in the solubility, viscosity, swelling, and stability of tamarind gum. TG and CMTG has been used in the development of various drug delivery systems[11–16]. Considering above facts there is demand to explore physicochemical properties of TG in order to ensure its suitability as an excipient in development of drug delivery systems. Hence, an attempt was made to extract, modify and characterize tamarind gum.

2. MATERIALS AND METHODS:

Tamarind kernel powder was kindly gifted by Chhaya Industries, Barshi, Maharashtra (India). Tamarind seeds were purchased from local market. All other chemicals and solvents were supplied by Loba Chemie, Mumbai, Maharashtra (India).

2.1 Extraction of TG:

TG was extracted from the tamarind kernel powder available in the market. The 20 g of defatted tamarind seed powder was added to 200 ml of cold distilled water to prepare slurry. Slurry was then poured into 800ml of boiling distilled water containing citric acid (0.2 %). The solution was boiled for 20 min with stirring in a water bath. Resulting thin clear solution was kept overnight (24 h) so that most of the proteins and fibers settle out, following which the solution was centrifuged at 5000 rpm for 20 min. Supernatant liquid was separated and poured into the excess of absolute alcohol with continuous stirring (1:1). Precipitate was washed with 200 ml of absolute ethanol, diethyl ether, and petroleum ether and/or acetone and dried at 50-60°C for 10 h. Dried polymer was powdered, sieved and stored in a desiccator until further use[17,18]. Also, TG was extracted using tamarind seeds. Percent yield was calculated and recorded. Flow chart of TG extraction is given in Figure 1.

2.2 Characterization of TG:

Organoleptic evaluation of TG:

Separated gum was evaluated for color, odor, taste, fracture, and texture. TG was cream brown in color, odorless and tasteless with irregular in shape. TG was rough in touch, texture, and fracture.

Shape of TG particles:

TG particles were observed under the Motic microscope at 10X resolution.

Identification tests:

Identification tests for TG were performed as per the standard procedures.

Determination of solubility:

A 100 ml (1% w/v) suspension of polysaccharide was transferred into a blender jar and blended at low speed for 3 min. The suspension was transferred to a centrifuge tube and centrifuged for 15 min. A 50 ml aliquot of supernatant was taken into a pre-weighed petri plate and dried in hot air oven at 105°C until the constant weight was obtained. Percent cold water solubility was then calculated and recorded[19].

Determination of PH:

A weighed quantity of TG was dispersed in distilled water to get 1% w/v solution. The pH of the resultant solution was measured by using pH meter.

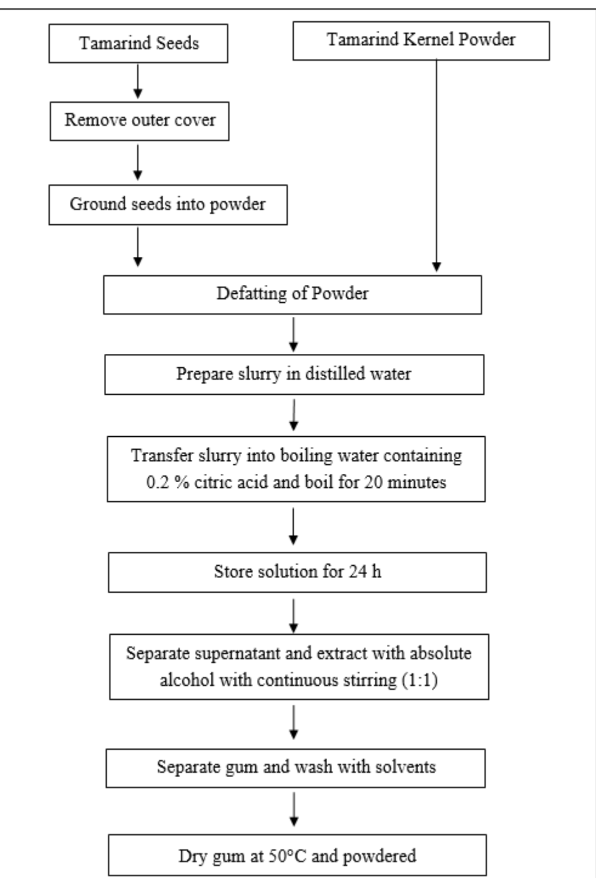
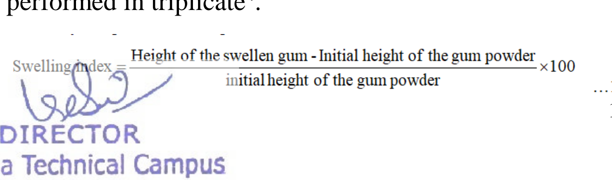


Figure 1: Extraction of TG.

Swelling index of TG:

Swelling profile of TG was determined by transferring accurately weighed 1 gm of TG into separate 25 ml measuring cylinders. The volume of each of cylinder was adjusted with solvent and observations were recorded for an increase in the volume of TG. Readings were taken at specified times until a constant volume was observed in each of the cylinders. The study was performed in triplicate[4].



$$\text{Swelling index} = \frac{\text{Height of the swellen gum - Initial height of the gum powder}}{\text{initial height of the gum powder}} \times 100 \quad \ldots 1$$

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Determination of viscosity:

Accurately weighed (1 gm) required quantity of TG was transferred to into separate 100 ml volumetric flask. These were made up to mark with distilled water. After one hour viscosity was measured. Small sample adapter (7ml) was used for measurement of viscosity with Spindle No. 21 rotated at 100 RPM. The study was performed in triplicate.

Bulk and tap density:

Accurately weighed 100 gm TG transferred into 250 ml graduated measuring cylinder and the initial volume of powder was recorded as V_0. The powder was subjected to 300 taps/min in tap density apparatus (Electrolab, Mumbai). The tapped volume was recorded as V_f. Bulk and tap density of TG were calculated using the following equations:

$$\text{Bulk density} = \frac{\text{Weignt of powder } (W)}{\text{Initial volume of powder } (V_0)} \quad (2)$$

$$\text{Tap density} = \frac{\text{Weignt of powder } (W)}{\text{Tap volume of powder } (V_f)} \quad (3)$$

***Carr's Index and Hausner's ratio*:**

Carr's index and Hausner's ratio are measures of the relative importance of interparticulate interactions. The Carr's index gives an idea about flowability of the powder. Carr's index can be calculated using the following equation:

$$\text{Carr's Index} = \frac{\text{Tapped density - Bulk density}}{\text{Tapped density}} \quad (4)$$

Hausner's ratio was calculated using the equation below:

$$\text{Hausner's ratio} = \frac{\text{Bulk density}}{\text{Tapped density}} \quad (5)$$

ATR-FTIR study of TG:

Infrared spectrum of was obtained using ATR-FTIR spectrophotometer (Shimadzu, Miracle 10, IR Affinity, Japan). The samples to be analyzed were placed onto the ATR and spectra were recorded in the range of 600–4000 cm^{-1} at an average of 25 scans and resolution of 4 cm^{-1}.

Thermal analysis of TG:

Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) of TG was performed using Mettler-Toledo TGA/DSC1 thermogravimetric analyzer (Mettler-Toledo, Switzerland). Samples were heated from 30ºC–300ºC at the rate of 10ºC/min, under a nitrogen atmosphere (flow rate: 10 ml/min).

Solid state ^{13}C NMR spectroscopy:

Solid state ^{13}C cross-polarization-magic angle spinning (^{13}C CP-MAS) NMR spectrum of TG was measured using JEOL-ECX400 spectrometer operating at 400 MHz (contact time of 3.5 ms, a relaxation delay of 5s, sweep width of 35 kHz and spinning speed of 10KHz). The chemical shifts were calibrated with the external hexamethylbenzene standard methyl resonance at 17.3 ppm.

X-ray powder diffraction:

X-ray diffraction (XRD) patterns of TG and CMTG were recorded using X-ray diffractometer (PW1729, Philips, The Netherlands) with a copper target, operated at voltage of 30 kV, 30 mA current, at 2°C/min scanning speed and scanning angle ranging from 0 to 90° (2θ).

2.3 Synthesis of CMTG:

Carboxymethylation of TG was carried out using the method reported by Goyal et al., 2007[20] (see Figure 2). TG (0.05 mol) was dispersed in 80ml alkaline aqueous methanol (0.158 mol sodium hydroxide). To this dispersion monochloroacetic acid (0.09 mol) was added in solid form with continuous stirring for 15 min. The flask was immersed in a thermostatic water bath and the temperature was maintained at 70°C for 60 min. The contents of the flask were shaken occasionally during the course of the study. The reaction product was filtered, dissolved in water and neutralized with dilute acetic acid. The reaction product was precipitated in ethanol and washed twice with aqueous methanol (80 %, v/v) followed by pure methanol. The product was initially dried at room temperature and then in a vacuum oven at 40°C for 4 h to obtain CMTG. The degree of substitution of CMTG was determined by titrimetric method[9,21].

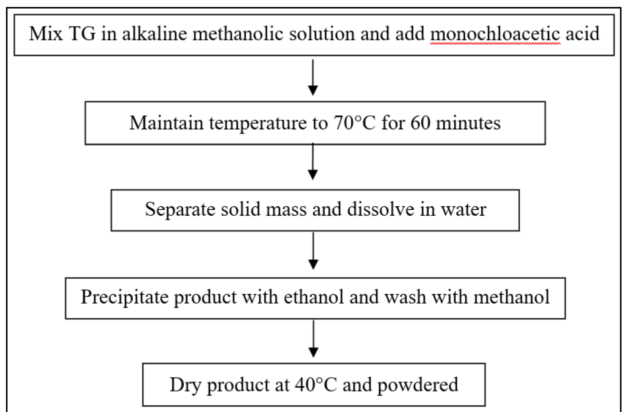

Figure 2: Synthesis of CMTG.

2.4 Characterization of CMTG:

Organoleptic properties, Shape of particles, identification tests, solubility, pH, swelling index, viscosity and powder characteristics of CMTG was as per procedure given in evaluation of TG. Further carboxymethylation of TG was confirmed by ATR-FTIR, thermal analysis and solid-state ^{13}C NMR of CMTG.

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3. RESULTS AND DISCUSSION:

3.1 Extraction of TG:

Results of extraction of TG are given in Table 1. Initially, TG was extracted from the tamarind seeds. The process to collect gum from tamarind seed was tedious, time-consuming and yield was also very low (less than 20%)[4]. This might be due to wastage of gum during extraction. Tamarind kernel powder is available in the market which contains fats, proteins, and carbohydrate. Fats present in tamarind kernel powder were removed with petroleum ether and was further used for extraction of TG. Tamarind kernel powder was added to boiling an aqueous solution of citric acid helps to separate proteins from the TG due to precipitation. TG present in the supernatant solution was separated by alcohol-precipitation[18]. The yield of TG was found to be more than 50% (~52.89±3.31%) when tamarind kernel powder was used for extraction. Obtained TG was passed through Sieve No 80 and stored in desiccator until further use.

Table 1: Extraction of TG

Parameter	Tamarind seeds	Tamarind kernel powder
Weight of Raw material (g)	20	20
Yield (g)	3.54	10.47
Yield (%)	17.69	52.89

3.2 Characterization of TG:

Organoleptic evaluation of TG:

Separated gum was evaluated for color, odor, taste, fracture, and texture. TG was cream brown in color, odorless and tasteless with irregular in shape. TG was rough in touch, texture and hard.

Shape of TG particles:

TG powder was observed under a Motic microscope at 10X resolution. Shape of particles is shown in Figure 3.

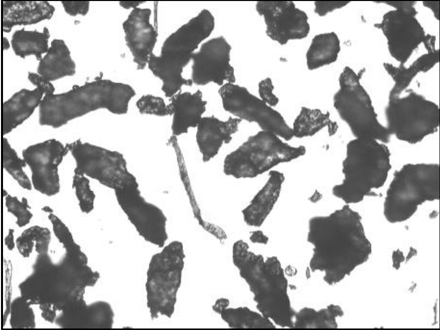

Figure 3: Microscopic image of TG.

TG particles were found to be irregular in shape with a rough surface. Most of the particles were of rectangular in shape. This may be due to the formation of thread-like structure during the extraction process.

Identification tests:

Results of identification tests are given in Table 2. Identification tests showed the presence of carbohydrate in the TG powder. When TG was mixed with Molisch's reagent followed by addition of sulfuric acid the violet color ring was appeared at the junction of the mixture in a test tube which confirms the presence of carbohydrate. TG powder showed negative test results for alkaloids, tannins, proteins, fats, and mucilages. This can be considered as proof for the purity of the isolated TG and free from proteins and fats.

Table 2: Chemical characterization of TG

Test	Present (+) /Absent (-)
Carbohydrate	+
Hexose sugar	+
Monosaccharides	-
Alkaloid	-
Tannins	-
Fats and oils	-
Proteins	-
Amino acids	-
Mucilages	-

Determination of solubility:

Cold water solubility of the TG sample was found to be 1.59±0.16 mg/ml. When the sample was heated it forms viscous solution due to swelling of TG in water. It indicates TG may form a gel. TG powder was found to be insoluble in ethanol, methanol, benzene, ether, and acetone.

Determination of pH:

pH of 1% TG in distilled water was found to be 6.52±0.18. It indicates that the TG is slightly acidic in nature.

Swelling of TG:

Swelling of TG was found to be 1.6 times of the dry volume of gum. It indicates TG can be used in sustained or controlled drug delivery of drugs.

Determination of viscosity:

Viscosity of 1 % TG was found to be 38.53±2.21 cP. It indicates a high concentration of TG is required to produce a gel.

Powder characteristics of TG:

Micromeritic properties of TG powder are given in Table 3. The flow properties of the powder material are dependent on the shape of the particles. It indicates that the prepared TG may be suitable for the development of solid dosage forms with the addition of suitable glidant.

Table 3: Micromeritic properties of TG

Parameter	Value
Bulk density (g/ml)	0.48
Tap density (g/ml)	0.57
Carr's Index (%)	15.8
Hausner's ratio	1.19
Angle of repose (°)	31.4

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ATR-FTIR study of TG:

ATR-FTIR spectrum of TG is given in Figure 4. ATR-FTIR spectrum of TG exhibited broad strong peaks at 3500–3000 cm^{-1} belonging to stretching vibration of –OH groups present in glucose, xylose and galactose units in the polysaccharide. A strong peak at 1039 cm^{-1} and 1143 cm^{-1} are attributed to the C-O stretching vibration of alcoholic group. The medium peak at 2920 cm^{-1} belonged to asymmetric stretching of CH. The peaks at 1747 cm^{-1} and 1689 cm^{-1} were due to carbonyl (-HC=O) stretching[22].

Thermal analysis of TG:

TGA-DSC of TG is given in Figure 5. Thermal decomposition curve of TG showed two main stages of decomposition. The first stage begins at 35°C and ends at 100°C. This may be due to the removal of free and bound water from the polymer. The second stage of weight loss was observed around 228°C to 300°C with 35% loss of weight. DSC thermogram of TG showed endotherm at 238.56°C. DSC curve supports the weight loss as evident in TGA curve.

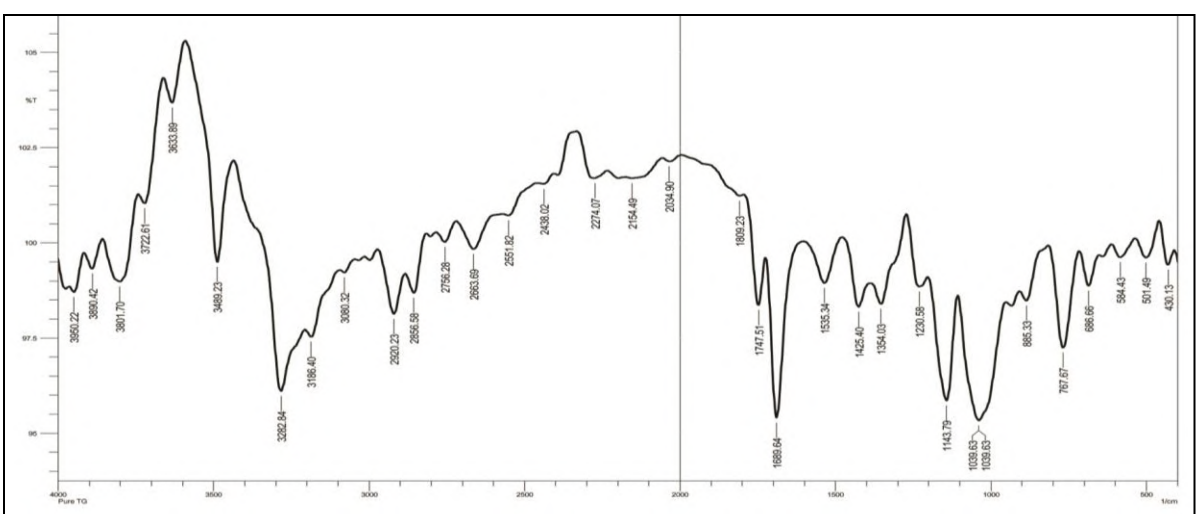

Figure 4: ATR-FTIR spectrum of TG.

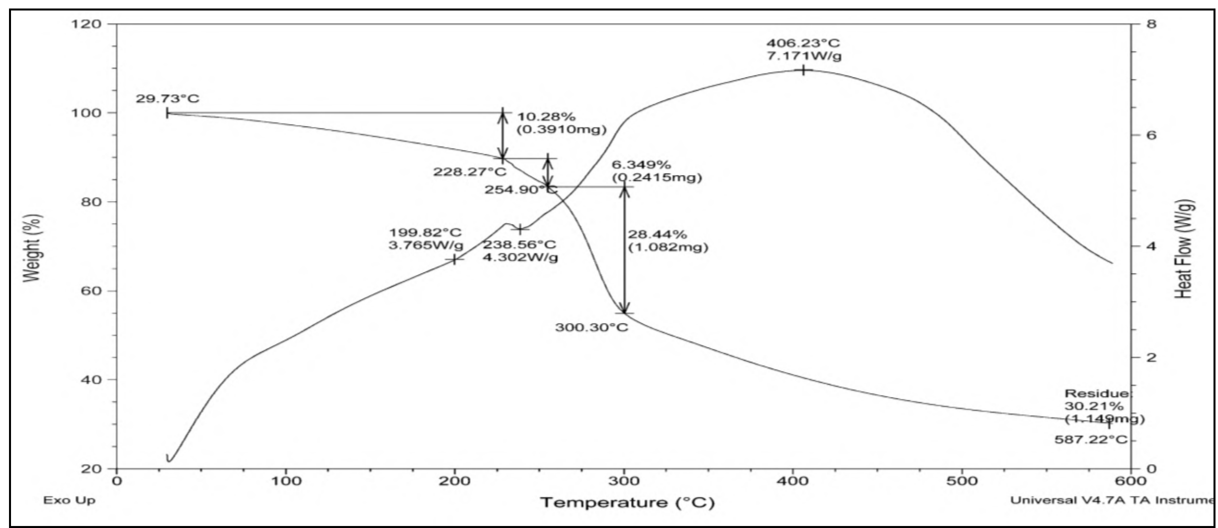

Figure 5: TGA-DSC of TG.

Solid state ^{13}C NMR spectroscopy:

Solid-state ^{13}C NMR spectrum of TG showed three distinct peaks (Figure 6). The resonance peak at 105ppm is assigned to an anomeric carbon atom (C1) and the peak at 74ppm is assigned to the carbon atoms (C2 to C5) connected by –OH groups (i.e., the carbon atoms in the six-membered ring except for C1 carbon atom). The presence of a peak at 63ppm is attributed to the C6 carbon atom of CH_2OH group.

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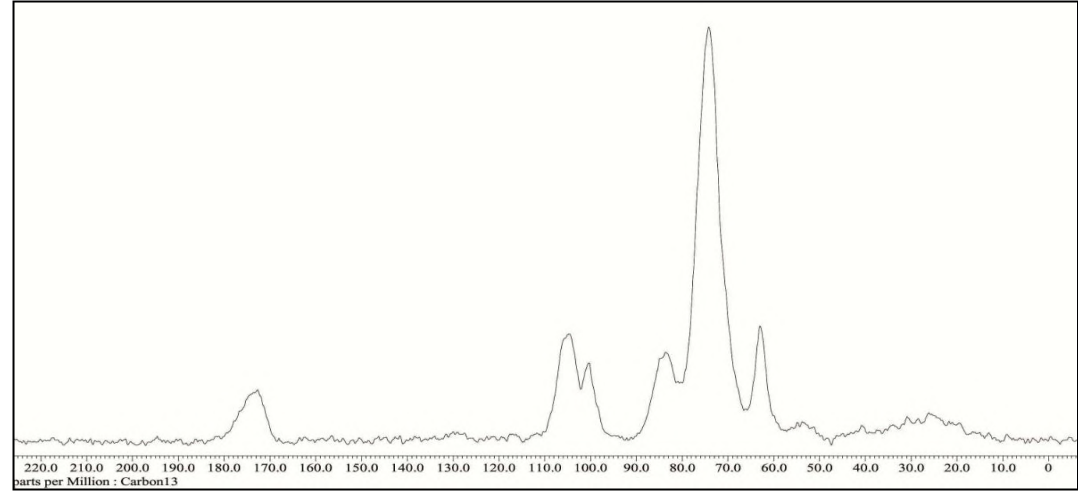

Figure 6: Solid state ^{13}C NMR of TG.

X-ray powder diffraction:

XRD of TG is given in Figure 7. TG did not show any characteristic peak, which indicates that the structure is completely amorphous.

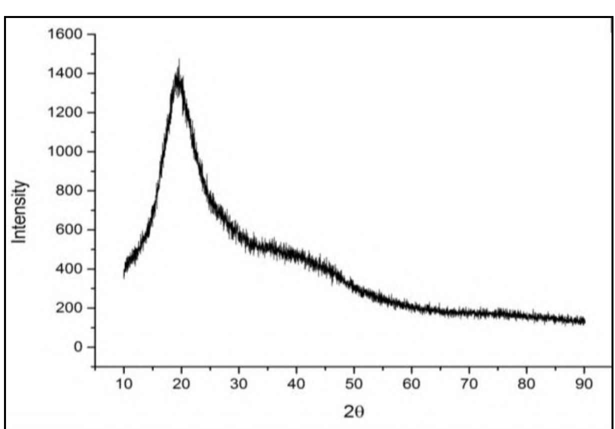

Figure 7: XRD of TG.

3.3 Synthesis of CMTG:

Addition of TG in a methanolic sodium hydroxide solution leads to the formation of TG-alkoxide. When this solution is heated in presence of monochloro acetic acid, a SN_2 reaction takes place in between TG-alkoxide and monochloro acetic acid resulting in the carboxymethylation of TG[20]. The carboxymethylation of TG was confirmed by the infrared spectroscopy. Batch size for carboxymethylation was 50 gm. The % yield of carboxymethyl TG was found to be 50.6±4.17 %. The degree of substitution was calculated using the titrimetric method and was found to be in the range of 0.16 to 0.2.

3.4 Characterization of CMTG:

Organoleptic evaluation of CMTG:

Sample of CMTG was evaluated for color, odor, taste, fracture, and texture. CMTG powder was light cream brown in color, odorless and tasteless with irregular in shape. CMTG was found to be rough in touch, texture, and fracture.

Shape of CMTG particles:

Microscopic image of CMTG is given in Figure 8. CMTG particles were found to be irregular in shape.

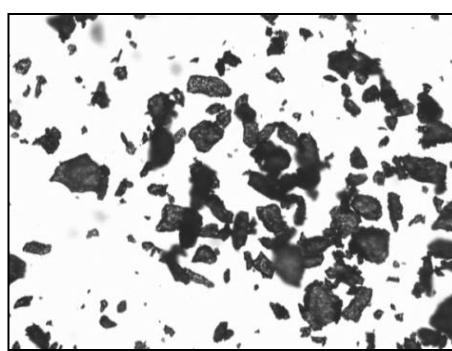

Figure 8: Microscopic image of CMTG particles.

Identification tests:

Test for carbohydrate was found to be positive and all other remaining tests were negative. It indicates synthesized CMTG was free from any other impurities.

Determination of solubility:

Cold water solubility of CMTG sample was found to be 10.53±1.28mg/ml. It indicates carboxymethylation of tamarind increases the solubility of tamarind gum. The sample of CMTG was found to be insoluble in ethanol, methanol, acetone, and benzene.

Determination of pH:

pH of 1% CMTG sample was found to be 5.94.

Swelling index of CMTG:

Swelling of CMTG was found to be two times of the dry volume of TG. It indicates CMTG can be used in sustained or controlled drug delivery of drugs.

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Determination of viscosity:
Viscosity of 1% CMTG was found to be 167.66±2.5 cP. Carboxymethylation of tamarind gum increased the viscosity of tamarind gum indicated the suitability of tamarind gum as matrix former as well as release retardant in development of novel drug delivery systems.

Powder characteristics of CMTG:
Powder characteristics of CMTG are given in Table 4. Carboxymethylation of tamarind gum improved the compressibility and flow properties of tamarind gum. This might be due to change in particle properties of CMTG. It suggests the suitability of CMTG in the development of solid dosage forms like tablets.

Table 4: Micrometric properties of CMTG

Parameter	Value
Bulk density (g/ml)	0.79
Tap density (g/ml)	0.85
Angle of repose (°)	24.94
Carr's index (%)	7.05
Hausner's ratio	1.07

ATR-FTIR of CMTG:
The ATR-FTIR spectrum of CMTG is given in Figure 9. The spectrum of CMTG exhibited broad strong peaks in the range of 3500 –3000 cm^{-1} representing stretching vibration of –OH groups present in glucose, xylose and galactose units in the polysaccharide. The medium peaks at 2856 cm^{-1} and 2927 cm^{-1} indicated asymmetric stretching of CH. The existence of a peak at 1745.56 cm^{-1} ascribed to C=O of the ester group. The peaks at 1639 cm^{-1} and 1402 cm^{-1} revealed the presence of carboxyl groups in CMTG. The existence of a peak at 1010 cm^{-1} indicated C-O-C stretch of the glycosidic link of CMTG[23,24]. The carboxymethylation is confirmed by the appearance of characteristic C=O and –COO bands at 1745.58 cm^{-1} and 1402 cm^{-1} respectively.

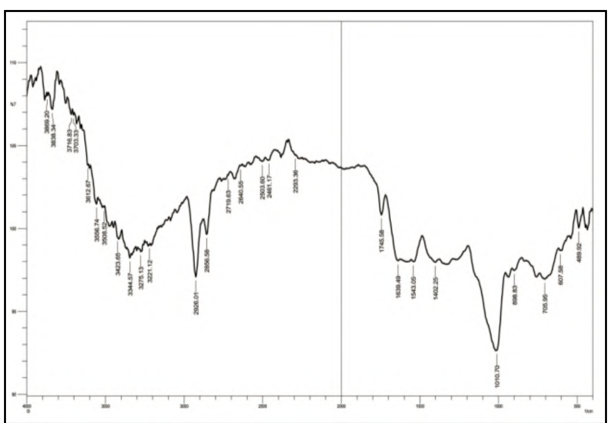

Figure 9: ATR-FTIR spectrum of CMTG.

DSC-TGA of CMTG:
DSC-TGA of CMTG is given Figure 10. Thermal decomposition curve of CMTG shows two main stages of decomposition. The first stage begins at 35°C and ends at 100°C with 3.91 % weight loss. This may be due to the removal of free and bound water from the polymer. The second stage of weight loss was observed between 235°C to 425°C with 54.42 % loss of weight. The total weight loss was found to be 90 % at 485°C. The weight loss of CMTG in the second stage is attributed to the decomposition of the polymer backbone. DSC curve supports the weight loss as evident in TGA curve.

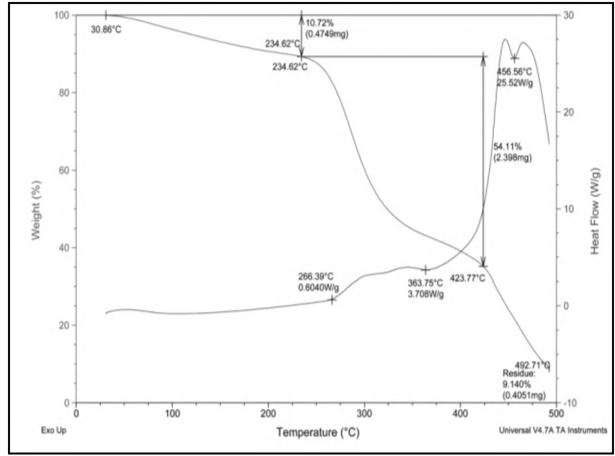

Figure 10: DSC-TGA of CMTG.

Solid state ^{13}C NMR of CMTG:
The solid-state ^{13}C NMR spectrum of CMTG shows three distinct peaks (Figure 11). The resonance peak at 105.2 ppm is assigned to the anomeric carbon atom (C1) and the peak at 74.28 ppm is assigned to the carbon atoms connected by –OH groups (i.e., the carbon atoms in the six-membered ring except for C1 carbon atom). The presence of a peak at 63.59 ppm corresponds to the C6 carbon atom of CH_2O- group. The signal at 173.32 ppm represents carbonyl carbon of CMTG. The solid-state ^{13}C NMR of hydrogel film shows all resonance peaks observed in CMTG[23].

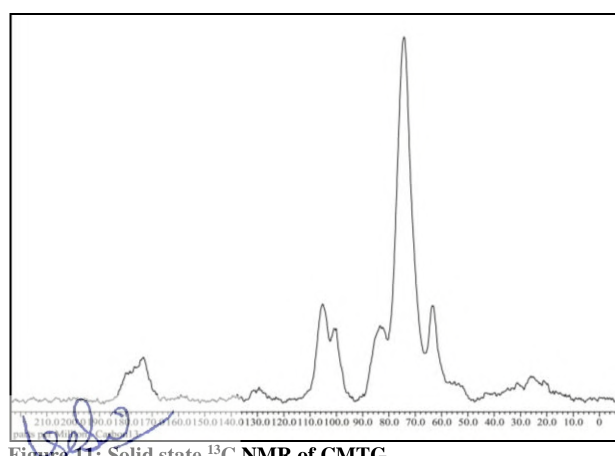

Figure 11: Solid state ^{13}C NMR of CMTG.

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X-ray powder diffraction:

XRD of TG is given in Figure 12. CMTG did not show any characteristic peak, which indicates that the structure is completely amorphous.

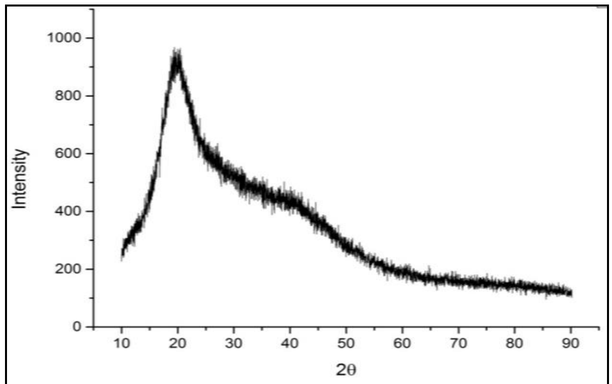

Figure 12: XRD of CMTG.

4. CONCLUSION:

Tamarind gum was successfully extracted with yield more than 50%. Extracted gum was free from impurities like proteins and fats. Physicochemical properties of tamarind gum indicate that it can be used as a pharmaceutical excipient in development of drug delivery. Functionalization of TG by carboxymethylation indicated improvement in physicochemical properties. Results of characterization revealed that carboxymethyl derivative can be potentially used for development of various drug delivery systems. It can be concluded that the TG and CMTG can be promising pharmaceutical excipients for the pharmaceutical industries.

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6. CONFLICT OF INTERESTS:

All authors approve the final manuscript and declare that there are no conflicts of interests.

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Utilization of Press mud for Improvement of Strength of Interlocking Bricks

Mr. Shaikh. A. N.[1], Miss.Deshmukh P.S.[2], Mr. Shelar V.E.[3]

[1,2,3] Asst. Professor, Department of Civil Engineering, Dnyanshree Institute of Engineering and Technology,Sajjangad Satara, Maharashtra, India.

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Abstract -Bricks are considered to be the most widely used and useful material of construction all over the world. In recent years, Interlocking brick has made significant advances which have resulted in economical improvements in strength of clay bricks. The economic development of nation depends upon the intelligent use of locally available materials. To overcome the use of natural resources and by attempting to use waste materials like waste Press-Mud and waste Fly ash in bricks could result in low cost construction. Waste Press mud is the byproduct of sugarcane factory which causes environmental problems because of its high production and less use. Sugar industries produce the huge amount of press mud and other waste materials. The production of press mud is significantly increased, due to increasing of the production of sugar and increasing the generation of new sugar factories also the use of waste press mud in manufacturing of interlocking bricks could help to avoid the problems related to environment pollution. An attempt has been made in this study to determine the maximum compressive strength of interlocking brick by using press mud consisting of sugarcane waste as partial replacement for fine aggregates (Grit). By using press mud, we can reduce the self-weight of brick. Interlocking bricks are used for easy construction of mortar less masonry and better appearance.

Key Words: Sugarcane Waste, Press Mud, Interlocking Brick, Mortar, Fly Ash, Grit.

1. INTRODUCTION

There is a strong demand for environmentally safe reuse and effective disposal method sugarcane press mud due to the increasing amount of sludge generated by various industries and plants in India. Landfills are commonly used for disposal of sludge in India, rapid urbanization has made it increasingly difficult to find suitable landfill sites. Therefore, incineration has become one of the few alternatives available for disposal of sugarcane press mud. The ultimate disposal of incinerated press mud can be accomplished by using it as engineering construction material. One possible solution for the management of this sugarcane press mud is to re-use it as a building material, namely, to incorporate this sugarcane waste press mud into interlocking bricks. The cement interlocking brick is a one of the most useful masonry building materials. The recycling of waste materials by incorporating them into interlocking bricks has been a popular topic of investigation over the last century, with varying degrees of success across a wide range of waste material of sugarcane press mud. This popularity is likely due to flexibility on the type of wastes which can be mixed into the brick making material, but more importantly, the high temperature involved in firing the bricks allows for the volatilization of dangerous Component, as well as the fixation of wastes into the vitreous phase of the brick. The current study investigates the potential for reusing sugarcane press mud by using it as a partial replacement of material [1].

1.1 What is Sugarcane Press Mud?

India is the second largest producer of the sugar in the world, with an annual output of 25 million tonnes. Among the steps leading to the production of refined sugar is the separation of sugarcane juice from the associated particulates. Upon this separation a solid residue is obtained which is called the press mud. In a typical sugar factory, the processing of 100 tonnes of sugarcane produces about 3 tonnes of press mud. In Maharashtra some sugar factory's 8–10 million tonnes of press mud are generated annually [2].

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1.2 COMPOSITION OF PRESS MUD:

Press mud from the sugar industries is a very useful source of fertilizer as well as some substances. The major use that has recently been developed in India is in bio composting (usually trade named as Bio earth) where it is treated with the spent wash from the distillery. The composition of press mud is given in Table-1. Its usefulness as fertilizer is based on the nutrient content of the press mud and the spent wash as shown below: [2].

Table -1: Composition of press mud.

Sr. No.	Composition	(%)
1	Crude wax	5-14
2	Fiber	15-30
3	Crude protein	5-15
4	SiO	4-10
5	CaO	1-4
6	PO	1-3
7	MgO	0.5-1.5
8	Total ash	9-10

Table -2: Nutrientcontentsof press mud.

Composition	Press mud	Spent wash (mg/l)
Nitrogen	1.15 – 3.0	2630
Phosphorus	0.60 – 3.50	201
Potassium	0.30 – 1.80	222

1.3 GENERATION OF PRESS MUD:

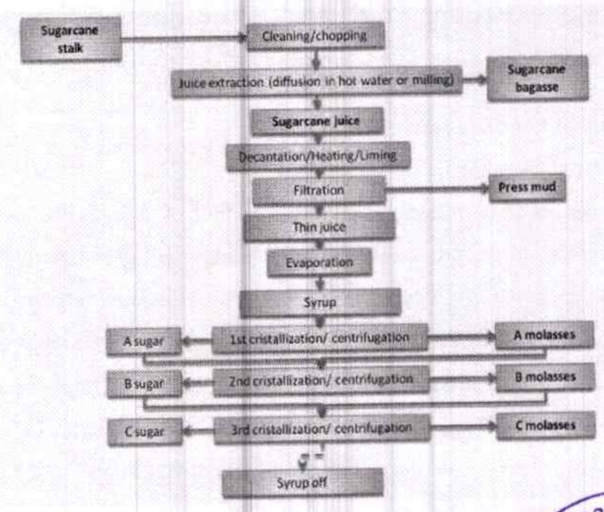

Fig -1: Generation of press mud.

2. LITERATURE REVIEW:

The waste from the industries is very harmful for the environment and also to our health, if not disposed in proper manner. The solid residue of sugarcane after crushing, extraction of its juice and before crystallization of s ugar.is known as “press mud”. India is one of the largest agriculture residues in the world [2]. The one way to dispose this waste is its use as fertilizer. But this is suitable for particular crops only. So, farmers avoid using it. The use of Sugarcane waste in brick can save the sugarcane industry disposal costs and produce an ecofriendly brick for construction. Sugarcane crop cultivation in India forms an important part of the Indian agricultural economy. The press mud can be used to recover protein, sugar and wax from press mud.

3. METHODOLOGY:

For the analysis purpose various interlocking brick samples are casted as per mix design with different percentage of sugarcane press mud and. the whole analysis is done in eight step which is given below.

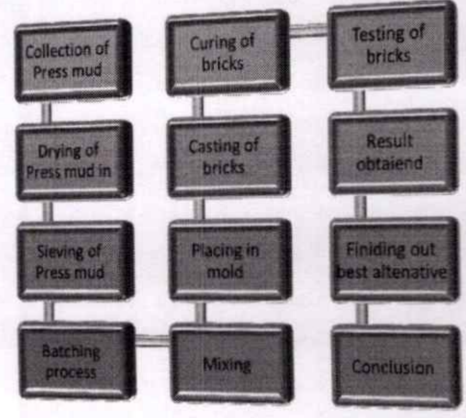

Chart -1: Process chart

3.1 COLLECTION OF PRESS MUD FROM FACTORY SITE:

Fig -2: Collection of press mud

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3.2 DRYING OF PRESS MUD IN SUN:

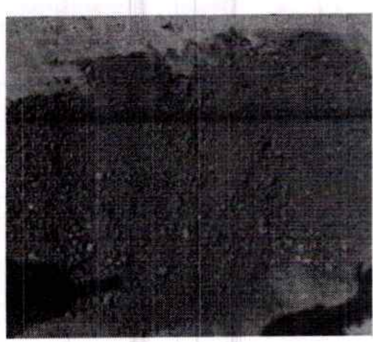

Fig -3: Drying of press mud

3.3 CASTING OF BRICKS:

Fig -4: Casting of bricks

3.4 CURING OF BRICKS:

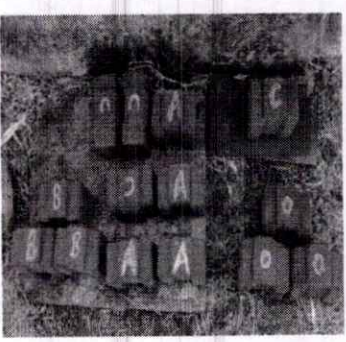

Fig -5: Placed for Curing

4. TEST ON BRICKS:

4.1. SHAPE AND SIZE TEST:

In this test, a brick specimen is closely inspected. It should be of standard size and its shape should be correctly rectangular with sharp edges. For this test, 3 bricks are selected at random and they are stacked length wise, along the width and along the height.

Results observed are:

1. One brick has not proper sharp edge.
2. Shape of brick slightly change due to breaking of edges.

4.2 WATER ABSORPTION TEST:

A brick is taken and it is weighted when it is dry. It is then immersed in water for a period of 24 hours. The brick is weighed again. The difference in weight indicates the amount of water absorbed by the brick. It should not exceed 20 percent of weight of dry brick.

Table -3: Water Absorption Test Result

Sr. No.	Block Name	Water absorption (%)
1	O	17.50 %
2	A	20.50 %
3	B	22.83 %
4	C	26.00 %

4.2 COMPRESSIVE STRENGTH TEST:

In this test the brick specimens are immersed in water for 24 hours. The specimen O, A, B, C is placed in compression testing machine. Then the load is applied axially at a uniform rate of 10 N/mm^2.The load and strength are noted accordingly.

Table -4: Compressive Strength Test Result

Sr. No.	Block Name	Compressive strength N/mm^2
1	O	3.54
2	A	4.16
3	B	3.75
4	C	2.93

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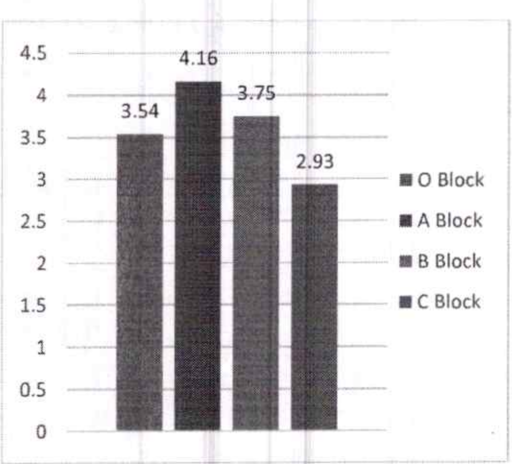

Chart No.2: Comparison of Compressive Strength Result N/mm^2 (28 Days)

5. CONCLUSIONS

Based on the above experimental procedure and test, we conclude as:

1. Use of sugarcane press mud in brick has solved the disposal problem; reduced cost and produced Eco- friendly brick for construction.
2. Reduction of weight of interlocking brick up to 20 % of weight of brick. As compere to normal interlocking bricks the bricks are light weight bricks.
3. In the Compressive strength result observed that block A is Shows moderately effect in increasing strength as compared to conventional brick i.e. greater than 3.5 N/mm^2.

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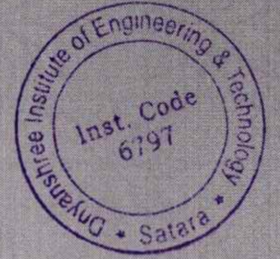

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