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Journal of Pharmaceutical

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MESSAGE





Our University proved its excellence in the areas of commencement of new courses and programmes, updating curriculum and all other academic related activities such as timely conduct of examinations and announcement of results. With Post Graduate and Ph.D courses in all the disciplines, research was given the due orientation by the university. Research grants were also sanctioned to encourage the fellow researchers, but the publication of research was still in its infancy. Marking the16th Year of its inception, it gives me immense pleasure to announce the year 2011-2012 as the year of "Journal publication" in different disciplines.

Continuous surveillance of research activities in the affiliated health institutions of the university, led us to the thought of publication of quality research under one roof. The implementation of the idea was publication of university's first Journals in Dental as well as Medical Disciplines. These publication received applaud from Academics, Professional and Faculties. The Student Fraternity was not only benefitted but also received encouragement from their own university to move in the direction of publishing their research work. Further, the thought process of publication of journals proliferated into other disciplines and the result is "RGUHS - Journal of Pharmaceutical Sciences" (RJPS). I hope the reader will embrace this publication also and utilize the opportunity to update and upgrade their knowledge. I wish this attempt of publication of scientific work; will also open up a platform for exchange of knowledge within the various disciplines of RGUHS.



Stat & Marin

Dr. S. Ramananda Shetty Vice-Chancellor RGUHS, Karnataka

Dear Readers,

Rajiv Gandhi University of Health Sciences Journal of Pharmaceutical Sciences, is a new Journal for the pharmaceutical sciences including life sciences, published by Rajiv Gandhi University of Health Sciences. This journal is not beholden to commercial interests or to a scientific society and is therefore free to respond to the needs of authors and readers and offers a truly cost-effective publishing service.

RJPS, a quarterly publication, caters for both specialized and interdisciplinary interests and covers all fields related to pharmaceutical sciences. The journal presents research articles and review articles depicting the present status and trends in Pharmaceutical Sciences and related disciplines including Life Sciences. Articles of particular interest covering the areas of pharmaceutical research, teaching & learning, laboratory innovations, education technology, curriculum design, examination reforms, training and other related issues will also be covered.

Other notable features of RJPS include fair and transparent evaluation procedures used for acceptability and a double-blind refereeing system. The journal publication is free of cost. This creates the ideal opportunity to try this journal and enjoy the benefits of publishing at no cost.

The editorial board along with a large reviewer panel and an experienced advisory board will be involved in evaluating the papers. The members of advisory board and the reviewer panel are drawn from all over India and abroad with proficiency in pharmaceutical sciences and allied fields. I can assure you of a prompt, courteous and unbiased assessment of your manuscript based on clearly stated criteria of acceptability.

I am delighted to welcome you to the launch of Rajiv Gandhi University of Health Sciences Journal of Pharmaceutical Sciences. I wish to extend my gratitude to the entire team, Associate editor, Executive editor, the Editorial board members, Reviewer panel and Advisory board as they are the backbone of this scientific endeavor.

> Editor-in-Chief Prof. B. G. Shivananda Principal Al-Ameen College of Pharmacy Bangalore



Dear Readers,

"If we knew what it was we were doing, it would not be called research, would it?" – an evocative quote by Albert Einstein

Man could well have originated science communication with the early discoveries. Science journalism is the key to the real treasure of the scientific knowledge, by virtue of which scientific knowledge and concepts could be carried to the common man. Thus the common man is benefitted with the new advancements in science and technology.

The literature of science is as much about wrong turns, aborted lines of query, failure to flourish, and outright failure as success. Even the greatest of scientists have failed now and again, just like the rest of us. Indeed, unless one is selective to the point of unrepresentativeness, it must be admitted that much of past science is a collection of dead ends and well-intentioned mistakes, eventually to be discarded. These failures can take many general forms. They can be discoveries that after a short time are proved erroneous, they can also be concepts with a long, successful track record that are found to be invalid or somehow theoretically inadequate, they can also be ambitious research programs that appear, from the vantage of observation, to have been futile because the thencurrent body of knowledge or research techniques were too primitive to permit a major breakthrough. Of course experiments, observations, or theories can be imperfect in some small or large way as error is human and part of the discovery process. Ultimately, scientists should be responsible for communicating research findings to the public. This communication is achieved by Journal articles.

Journal articles are an integral form of scientific discourse and the most common form of scientific communication. Over the centuries, five types of scientific articles have emerged: theoretical, experimental, observational, methodological, and review. They are distinct, each having a different purpose, though closely intertwined. Theoretical articles focus on explaining natural events, often suggesting experiments or observations that might confirm the explanations. They offer the new conceptual variations that drive the continued evolution of science. Experimental articles recount the manipulations of natural objects, usually in artificial settings such as research laboratories. They provide the empirical information essential for the continued conceptual evolution of science. Observational articles describe natural objects, usually outside the laboratory. They do not primarily involve manipulating natural objects under controlled conditions. Observational articles complement the experimental; they exist because part of the task of science will always involve describing the natural world outside the laboratory. Methodological articles do not usually make new claims but present new means for facilitating and creating experiments and improving observations. They are about the tools used to create new science. Review articles describe and evaluate the recent literature in a field; they usually contain no major claims not presented in previous articles. While their purpose is to interpret past science, not invent new science, they serve an indispensable function--winnowing the fit from the unfit among the other four types. They constitute a second tier of peer review, one far more selective than the first.

In more than 50 pharmacy colleges affiliated to RGUHS, research is a part of academic curriculum. Under the guidance and dynamic leadership of our Vice Chancellor, an attempt is being made to publish quality research, as scientific publication. The RGUHS Journal of Pharmaceutical Sciences is a broad-spectrum, peer-reviewed, quarterly publication, compiles, research articles and review articles depicting the present status and trends in Pharmaceutical Sciences.

Executive Editor Dr. Roopa S. Pai Professor of Pharmaceutics Al-Ameen College of Pharmacy, Bangalore,

Design and Evaluation of Atenolol Bilayer Buccal Tablets

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- In the present study, mucoadhesive buccal bilayer tablets of atenolol were fabricated with objective of avoiding first pass metabolism and improve its bioavailability with reduction in dosing frequency. Bilayer buccal tablets of atenolol were prepared by direct compression method using combination of polymers such as hydroxypropyl methylcellulose 15 cps
- and 50 cps along with Carbopol 934p and ethylcellulose as backing layer. The double layer structure design was expected
- to provide drug delivery in a unidirectional fashion to the mucosa and avoid loss of drug due to washout with saliva. The
- \prec designed tablets were evaluated for various physical and biological parameters.
- The formulation BT₁ containing hydroxypropyl methylcellulose 15 cps (48% w/w of matrix layer), Carbopol 934p (2%
- ▶ w/w of matrix layer) and mannitol (channeling agent, 14% w/w of matrix layer) was found to be promising. This
- o formulation exhibited an *in vitro* drug release of 86.45% in 9 h along with satisfactory bioadhesion strength (5.30 g).
- Short-term stability studies on the promising formulation indicated that there are no significant changes in drug content
- and in vitro dissolution characteristics (p<0.05). IR spectroscopic studies indicated that there are no drug-excipient interactions. The prepared buccal tablets of atenolol were able to stay in the buccal cavity for a longer period of time, which indicates a potential use of mucoadhesive tablets of atenolol for treating blood pressure.

Keywords: Mucoadhesive buccal tablet, atenolol, swelling index, bioadhesive strength.

INTRODUCTION

Among the various routes of drug delivery, the oral route is perhaps the most preferred by patients and clinicians alike. However, peroral administration of drugs has disadvantages, such as hepatic first-pass metabolism and enzymatic degradation within the gastrointestinal tract (GIT). So, there has been a growing interest in the use of delivery of therapeutic agent through various transmucosal routes to provide a therapeutic amount of drug to the proper site in body to promptly achieve and then maintain the desired concentration. Consequently, other absorptive mucosa are considered as potential sites for drug administration. Transmucosal routes of drug delivery (i.e. the mucosal linings of the oral, nasal, rectal, vaginal, and ocular cavities) offers distinct advantages over peroral administration for systemic effect.¹

The unique environment of the oral cavity offers its potential as a site for drug delivery. These advantages of this route of administration include: 1) The drug is not subjected to the destructive acidic environment of the stomach. 2) Therapeutic serum concentration of the drug can be achieved more rapidly and 3) The drug enters the general circulation without first passing through the liver.

The mouth is lined with mucous membrane and among the

RGUHS Journal of Pharmaceutical Sciences Received: 07/01/2011, Modified: 22/02/2011, Accepted: 01/03/2011 least known of its functions is its capability of serving as a site for the absorption of drugs.² In general, drugs penetrate the mucous membrane by simple diffusion and are carried in the blood, which is richly supplied with the salivary glands and their ducts, into the systemic circulation via the jugular vein. Active transport, pinocytosis and passage through aqueous pores usually play only insignificant roles in moving drugs across the oral mucosa.³ Two sites within the buccal cavity have been used for drug administration. Using the sublingual route, the medication is placed under the tongue, usually in the form of rapidly dissolving tablet. The second anatomic site for drug administration is between the cheek and gingiva, although this second application site is itself known as buccal absorption.

The thin mucin film, which exists on the surface of the oral mucosa, may provide an opportunity to retain a drug delivery system in contact with the mucosa for prolonged period, if it is designed to be mucoadhesive. Such system ensures close contact with absorbing membrane, thus optimizing the drug concentration gradient across the biological membrane and reducing the differential pathway.

In addition it should release the drug in a unidirectional way towards the mucosa, in a controlled and predictable manner, to elicit the required therapeutic response. This unidirectional release can be achieved using bilayer device. Therefore, the oral mucosa may be used as a potential site for controlled or sustained drug delivery. From the technological point of view, an ideal buccal dosage form must have three properties; It must maintains its position in the mouth for a few hours, release the drug in controlled fashion and provide drug release in a unidirectional way towards mucosa.

Atenolol (beta blocker), has been widely used in the management of hypertension. The drug is well absorbed from the gastrointestinal tract but its bioavailability is low (54%) due to extensive first pass metabolism.⁴ Since the buccal route bypasses first-pass effect, the dose of atenolol could be reduced by 50%. The physicochemical properties of atenolol, its suitable half-life (6-7 h) and low molecular weight (266.34) makes it a suitable candidate for administration by buccal route. The effective permeation of the drug through bovine buccal mucosa has already been reported.⁵

In the present study, an attempt was made to design efficacious and prolonged release mucoadhesive tablets of atenolol using various polymers to avoid first pass metabolism, to reduce dosing frequency and to improve patient compliance with improved bioavailability.

MATERIALS AND METHODS

Atenolol was gifted by Rajat Pharmachem Ltd, Ankaleshwar, Gujarat. Ethyl cellulose was gifted by (Arihant Trading co., Mumbai, India), hydroxypropyl methylcellulose 15 cps, 50 cps, (Colorcon Asia Pvt. Limited, Verna, India) and carbopol 934p were gifted by (ShinEtsu Chemical Co. Ltd Japan). All other materials were of analytical or pharmacopoeial grade and used as received.

Preparation of the buccal tablets⁶⁻⁷

Preparation: Direct compression method has been employed to prepare buccal tablets of atenolol using HPMC 15cps, HPMC 50cps and Carbopol 934p as polymers. **Procedure:** All the ingredients including drug, polymer and excipients were weighed accurately according to the batch formula (Table 1). The drug is thoroughly mixed with mannitol on a butter paper with the help of a stainless steel spatula. Then all the ingredients except lubricant were mixed in the order of ascending weights and blended for 10 min in an inflated polyethylene pouch. After uniform mixing of ingredients, lubricant was added and again mixed for 2 min. The prepared blend (100 mg) of each formulation was pre-compressed, on a 10-station roatory tablet punching machine (Clit, Ahmedabad) at a pressure of 0.5 ton and turret speed of 2 rpm to form single layered flat-faced tablet of 9 mm diameter. Then, 50 mg of ethyl cellulose powder was added and final compression was done at a pressure of 3.5 tons and turret speed of 2 rpm to get bilayer tablet.

Evaluation of buccal tablets

The prepared batches of tablets were evaluated for weight variation, hardness, friability, drug content uniformity, swelling index, surface pH, *ex vivo* mucoadhesive strength, *in-vitro* drug release, short-term stability (IR spectroscopy) and drug-excipient interaction.

Twenty tablets were selected at random and weighed individually. The individual weights were compared with the average weight for determination of weight variation. Hardness and friability of the tablets were determined by using Monsanto hardness tester and Roche friabilator respectively. For content uniformity test, ten tablets were weighed and powdered. The powder equivalent to 25 mg of drug was extracted into methanol, filtered through 0.45 m membrane filter disc (Millipore Corporation) and was analyzed for atenolol after appropriate dilution by measuring the absorbance at 226.6 nm, against blank. The drug content was calculated using the standard calibration curve. The mean percent drug content was determined as an average of three determinations.

Table 1: Composition of Buccoadhesive Tablets										
Ingredients [*]	Formulation code									
(mg/tablet)	AT ₀	AT ₁	At_2	\mathbf{AT}_{3}	\mathbf{AT}_{4}	BT ₀	BT ₁	BT ₂	BT ₃	BT ₄
Atenolol	25	25	25	25	25	25	25	25	25	25
Carbopol 934P		2	4	8	12		2	4	8	12
HPMC 50 cps	50	48	46	42	38					
HPMC 15 cps						50	48	46	42	38
Mannitol	14	14	14	14	14	14	14	14	14	14
PVP K-30	6	6	6	6	6	6	6	6	6	6
Aspartame	3	3	3	3	3	3	3	3	3	3
Mg. stearate	2	2	2	2	2	2	2	2	2	2
Ethyl cellulose	50	50	50	50	50	50	50	50	50	50
Total weight	150	150	150	150	150	150	150	150	150	150
*Weights expressed a	s mg per	tablet; H	PMC- hy	droxypro	opyl metl	nylcellulo	se; PVP-	polyviny	l pyrolid	one;

AT-formulation containing HPMC 50 cps; BT-formulation containing HPMC 15 cps.

For the determination of surface pH of the buccal tablets, a combined glass electrode is used. The tablet is allowed to swell by keeping it in contact with 1 ml of distilled water (pH 6.8 ± 0.05) for 2 h at room temperature. The pH is identified by bringing the electrode into contact with the tablet surface and allowing to equilibrate for 1 min.⁸ (Fig.1).

The swelling index^{9,10} of the buccal tablet was evaluated by using pH 6.8 phosphate buffer. The initial weight of the tablet is determined (w₁). The tablet was placed in pH 6.8 phosphate buffer (6 ml) in a petri-dish placed in an incubator at 37 ± 1 °C and tablet was removed at different time intervals (0.5, 1.0 to 9.0 h), and reweighed (w₂) (Fig.2). The swelling index was calculated using the formula:

Swelling index = $100 (w_2 - w_1) / w_1$.

Mucoadhesion strength:¹¹⁻¹ The apparatus used for testing bioadhesion was assembled in the laboratory. Mucoadhesion strength of the tablet was measured on a modified physical balance employing the method described by Gupta et al¹⁴ using bovine cheek pouch as model mucosal membrane. (the buccal mucosa was collected from the local slaughter house).

A double beam physical balance was taken, the left pan was removed. To left arm of balance a thick thread of suitable length was hanged. To the bottom side of thread a glass stopper with uniform surface was tied. A clean glass mortar was placed below hanging glass stopper. In this mortar a clean 500 ml glass beaker was placed, within which was placed another glass beaker of 50 ml capacity in inverted position and weighed with 50 g to prevent floating. The temperature control system involves placing thermometer in 500 ml beaker and intermittently adding hot water in outer mortar filled with water. The balance was so adjusted that right hand-side was exactly 5 g heavier than the left.

Method: The balance adjusted as described above was used for the study. The bovine cheek pouch, excised and washed was tied tightly with mucosal side upward using thread over the base of inverted 50 ml glass beaker. This beaker suitably weighed was lowered into 500 ml beaker, which was then filled with isotonic phosphate buffer (pH 6.8) kept at 37° C such that the buffer reaches the surface of mucosal membrane and keeps it moist. This was then kept below left hand side of balance. The buccal tablet was then stuck to glass stopper through its backing membrane using an adhesive (Feviquick). The 5 g on right hand side is removed; this causes application of 5 g of pressure on buccal tablet overlying moist mucosa. The balance was kept in this position for 3 min and then slowly weights were increased on the right pan, till tablet separates from mucosal membrane. The total weight on right pan minus 5 g gives the force required to separate tablet from mucosa. This gives bioadhesive strength in grams. The mean value of three trials was taken for each set of formulations. After each measurement, the tissue was gently and thoroughly washed with isotonic phosphate buffer and left for 5 min before reading a new tablet of same formulation to get reproducible multiple results for the formulation (Fig. 3).

In vitro drug release study:¹⁵⁻¹⁶

This is carried out in USP XXIII tablet dissolution test apparatus-II (Electrolab TDT-06N), employing paddle at 50 rpm and 200 ml of pH 6.8 phosphate buffer as dissolution medium. The release study is performed at $37\pm0.5^{\circ}$ C. The backing layer of the buccal tablet is attached to glass disk with cyanoacrylate adhesive. The disk is placed at the bottom of the dissolution vessel. Samples of 5 ml are withdrawn at predetermined time intervals and are replaced with fresh medium. The samples were filtered through 0.25 m Whatman filter paper and analyzed for atenolol after appropriate dilution by measuring the absorbance at 226.7 nm. The experiment was run in triplicate.

Stability studies:

Accelerated stability studies were performed at a temperature of $40\pm2^{\circ}$ C / $75\pm5\%$ RH over a period of three months (90 days) on the promising buccal tablets of atenolol (formulations BT₁). Sufficient number of tablets (15) were packed in amber colored rubber stoppered vials and kept in stability chamber maintained at $40\pm2^{\circ}$ C / $75\pm5\%$ RH. Samples were taken at one month interval for drug content estimation. At the end of three month period, dissolution test was also performed to determine the drug release profiles.

Drug-Excipient Interaction Studies:

The IR spectra of atenolol, Carbopol 934P, HPMC 15cps, HPMC 50 cps, PVP K-30, magnesium stearate and formulations (BT_1) were obtained by KBr pellet method. (Perkin-Elmer series 1615 FTIR Spectrometer).

RESULTS AND DISCUSSION

It has been proposed that mucoadhesion occurs in three stages. The first stage involves the formation of an intimate contact between the mucoadhesive and mucous. Secondly, the mucoadhesive macromolecules swell and interpenetrate the mucus macromolecules, becoming physically entangled. Thirdly, these molecules interact with each other via secondary, non-covalent bonds such as hydrogen bonds.

The main goal of this work was to develop new buccoadhesive bilayer tablets of atenolol, an antihypertensive drug (beta blocker), consisting of drug free non-adhesive protective layer. The double layered structure design was expected to provide drug delivery in an

unidirectional fashion to the mucosa and to avoid loss of drug due to washout by saliva, release drug immediately to produce a prompt pharmacological action and remain in oral cavity and provide a sustained release of enough drug over an extended period of time. A total of ten formulations of buccoadhesive bilayer tablets of atenolol were prepared and evaluated for biological, physical and mechanical parameters. The blends were also evaluated for various pre compression parameters. These blends displayed angle of repose values of about 35°; bulk density, tapped density and Carr's index values were found approximately to the 0.35 g/cc, 0.41 g/cc and 14.63 % respectively. According to work plan, the tablets were evaluated for their thickness, hardness, friability, weight variation, swelling index, surface pH, drug content and mucoadhesive strength. The appearance of buccoadhesive tablets was smooth and uniform on physical examination. The hardness of prepared tablets of atenolol was found to be 3.2 to 4.6 kg/cm²; hardness increases with increasing Carbopol 934p proportion in the formulation. The thickness and weight variation were found to be uniform as indicated by the low values of standard deviation, and were found to be in the range of 2.97 to 3.05 mm and 147.9 to 150.6 mg respectively. Friability values less than 1% indicate good mechanical strength to withstand the rigors of handling and transportation. Results are given in (Table 2). The drug content of tablets was quite uniform as seen in the above mentioned table. The average drug content of the tablets was found to be within the range of 94.15% to 104.51 % and the low values of standard deviation and coefficient of variation (<1, not shown in the table) indicate uniform distribution of the drug within the prepared buccoadhesive tablets. The surface pH of all the tablets was within a range of 5.23 to 6.77 (Table 2) which was close to neutral pH. Hence it is assumed that these formulations cause no irritation in the oral cavity. The swelling profile of different batches of the tablets is shown in Table 2. The swelling state of the polymer (in the formulation) was reported to be crucial for its bioadhesive behaviour. Adhesion occurs shortly after the beginning of swelling but the bond formed between mucosal layer and polymer is not very strong. The adhesion will increase with the degree of hydration until a point where over-hydration leads to an abrupt drop in adhesive strength due to disentanglement at the polymer/tissue interface. Results indicate that as the concentration of Carbopol 934P increases the swelling index increases. The mucoadhesive strength of the tablets was found to be maximum in case of formulation AT, i.e. 6.6 gm. This may be due to fact that positive charges on surface of Carbopol 934P could give rise to strong electrostatic interaction with mucous or negatively charged mucous membrane.

In vitro **Drug Release**: From dissolution data it is evident that the designed formulations have displayed more than 57% drug release in 9 h. The formulation BT_1 containing hydroxypropyl methylcellulose 15 cps (48% w/w of matrix layer), Carbopol 934P (2% w/w of matrix layer), and mannitol (channeling agent, 14% w/w of matrix layer) was found to be promising, which showed $t_{25\%}$, $t_{50\%}$ and $t_{70\%}$ values of 0.33, 1.57 and 5.42 h respectively and released 86.45% drug within 9 h. Results are shown in (Table 3) and the drug release profiles depicted in figures 4 and 5. A comparison of the release parameters is shown in figure 6.

Drug Release Kinetics: In vitro drug release data of all the buccoadhesive tablet formulations of atenolol was subjected to goodness of fit test by linear regression analysis according to zero order, first order kinetics and according to Higuchi's and Peppas models to ascertain mechanism of drug release. It was evident that all the formulations displayed zero-order release kinetics (after an initial burst release of 14-28% drug, with 'r' values from 0.743 to 0.887). Higuchi and Peppas data reveals that the drug is released by non-Fickian diffusion mechanism ('r' values from 0.441 to 0.920 and 'n' values from 0.803 to 0.981). The IR spectrum of the pure drug atenolol displayed characteristic peaks at 3362.04 cm⁻¹ and 1636.69 cm⁻¹due to N-H and C=O amide groups respectively. The peaks of 1240.27 cm⁻¹ and 2972.40 cm⁻¹ are due to alkyl aryl ether linkage and alcoholic -OH groups respectively. All the above characteristic peaks were also found in the IR spectrum of the formulation BT_1 (peaks at 3356.12 cm⁻¹ and 1647.26 cm⁻¹due to -NH and C=O stretching respectively and peaks at 1244.11 cm⁻¹ and 2972.40 cm⁻¹ are due to alkyl aryl ether linkage and alcoholic -OH groups respectively). The presence of above peaks confirms undisturbed structure of drug in the above formulation. Hence, there are no drug-excipient interactions. The stability studies data indicates that the drug content of formulation BT₁ was not significantly affected at 40 ± 2 °C / 75±5% RH after storage for three months. The 't' value was found to be 1.03 against the table value of $4.3 \, (p < 0.05)$.

CONCLUSION

The results of the present study indicate that buccoadhesive bilayer tablets of atenolol with controlled drug release can be successfully prepared by direct compression method using HPMC 15 cps, HPMC 50 cps and Carbopol 934p as mucoadhesive polymers and ethyl cellulose as backing layer. It exhibited well controlled and delayed release pattern. This study concludes that, the addition of carbopol 934p increases the viscosity and swelling of tablets there by controls the release of drug and improves the mucoadhesive properties.

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			Table 2	:: Evaluation of Bu	uccal Tablets			
Formulation code	Mean Hardness* (kg/cm2)	Mean Thickness* (mm)	Weight Variation* (mg)	Friability (%)	Mean % Drug Content*	Surface PH*	Swelling Index* (after 9 h)	Mucoadhesive Strength* (gm)
At_{0}	3.4 ± 0.45	2.99 ± 0.05	148.1±0.88	0.79 ± 0.01	95.08 ± 0.62	6.23 ± 0.09	22.19±0.05	4.43±0.12
AT_1	3.9 ± 0.76	3.10 ± 0.06	147.9 ± 0.99	0.67 ± 0.01	94.15 ± 2.76	6.26 ± 0.09	34.58 ± 0.60	5.27 ± 0.15
AT_2	4.2 ± 1.02	$3.00{\pm}0.06$	150.6 ± 0.99	0.57 ± 0.01	101.09 ± 0.34	$5.76 {\pm} 0.06$	52.26 ± 0.97	6.23 ± 0.12
$\mathrm{AT}_{\scriptscriptstyle 3}$	4.4 ± 0.99	2.97 ± 0.06	149.6 ± 0.99	0.55 ± 0.00	97.23 ± 0.23	$6.54{\pm}0.06$	57.87 ± 0.98	6.43 ± 0.06
AT_4	4.6 ± 0.72	2.98 ± 0.00	150.1 ± 0.99	0.51 ± 0.01	97.09 ± 0.30	6.63 ± 0.10	70.69±0.64	6.60 ± 0.10
$\mathbf{BT}_{_0}$	3.2 ± 1.06	2.97 ± 0.06	150.7 ± 1.06	0.87 ± 0.03	97.75 ± 0.36	5.87 ± 0.12	29.54 ± 0.09	3.75 ± 0.07
${\operatorname{BT}}_1$	3.7 ± 0.85	2.97 ± 0.06	148.6±0.84	0.72 ± 0.98	96.38 ± 1.56	5.73 ± 0.08	40.90±0.89	5.30 ± 0.14
${\operatorname{BT}}_2$	4.5 ± 0.45	2.99 ± 0.06	148.7 ± 0.95	0.56 ± 0.02	102.73 ± 1.63	6.56 ± 0.95	42.55 ± 0.56	5.75 ± 0.21
BT_3	4.6 ± 0.54	2.99 ± 0.06	150.5 ± 0.95	0.52 ± 0.87	98.40 ± 0.84	5.23 ± 0.95	57.83 ± 0.99	5.95 ± 0.07
${\operatorname{BT}}_4$	4.6 ± 1.00	3.10 ± 0.00	150.2 ± 0.97	0.56 ± 0.67	104.51 ± 0.83	6.77 ± 0.97	78.04 ± 0.98	6.05 ± 0.07
		*Average of the Horm	hree determination	s, values shown ir decred as the best	n parenthesis are star and used for furthe	ndard deviations. r studies		





Fig. 3: Bioadhesion Testing Apparatus

Fig. 4: FTIR spectra of atenolol pure drug.



Fig. 6: *In vitro* drug release profiles of formulations using HPMC (50 cps) as mucoadhesive polymer





Fig. 8: comparison of dissolution parameters (t_{25} %, t_{50} % and t_{70} %) of buccoadhesive tablets of atenolol 8 7 6 5 4 Time(hrs) 3 2 0 AT1 AT2 AT4 BT0 BT1 BT2 BT3 BT4 AT0 AT3 Formulation Code t25% t50% t70%

Table 3: In Vitro Drug Release Parameters								
Formulation	$t_{25\%}(h)$	$\mathbf{t}_{_{50\%}}(\mathbf{h})$	t _{70%} (h)	Cumulative %				
code				drug release in 9 h * ±SD				
AT ₀	0.39	1.51	7.5	86.98±1.69				
AT_1	0.48	2.51	>9	63.26 ± 1.42				
AT_2	0.51	3.06	>9	62.13 ± 2.48				
AT_3	0.54	4.24	>9	58.33 ± 0.67				
AT_4	0.51	3.42	>9	57.14 ± 0.91				
BT ₀	0.30	1.48	4.12	93.68 ±2.11				
BT_1	0.33	1.57	5.42	86.45 ± 1.0				
BT_2	0.42	2.3	7.42	74.19 ± 3.57				
BT_3	0.45	3.18	>9	61.52 ± 3.55				
BT_4	0.48	3.46	>9	60.46 ± 2.22				
$t_{25\%}$, $t_{50\%}$ and $t_{70\%}$ are time for 25%, 50% and 70% drug release respectively; *Average of three determinations, SD-standard deviation.								

The formulation BT_1 containing hydroxypropyl methylcellulose 15 cps (48% w/w of matrix layer), Carbopol 934p (2% w/w of matrix layer), and mannitol (channeling agent, 14% w/w of matrix layer) was found to be promising, which shows an *in vitro* drug release of 86.45% in 9 h along with satisfactory bioadhesion strength (5.30 g).

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Evaluation of Disintegrating Properties of Mangifera indica gum

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In the present study, an attempt had been made to prepare mouth dissolving tablets of metformin HCl using *Mangifera indica* gum powder as disintegrant. Specifications for herbal raw materials and finished products were set according to Committee for Proprietary Medicinal Products. Gum extracted from the *Mangifera indica* tree was subjected to toxicity studies for its safety and preformulation studies for its suitability as a disintegrating agent. The gum extracted is devoid of toxicity. mouth dissolving tablets of metformin were prepared and compared with different concentrations viz; 2, 4, 6, 8 and 10 % (w/w) of *Mangifera indica* gum powder and crosspovidone[®], and evaluated for physical parameters such as thickness, hardness, friability, weight variation, drug content, disintegration time and drug dissolution. The physical parameters of the fabricated tablet were within acceptable limits. The formulated tablets had good appearance and better drug release properties. The study revealed that *Mangifera indica* gum powder was effective as disintegrants in low concentrations (6%w/w). The study further revealed a poor relation between the swelling index and disintegrating efficiency. Studies indicated that the extracted mucilage is a good pharmaceutical adjuvant, specifically a disintegrating

Keywords: Metformin HCl, Disintegrant, mouth dissolving tablets, Mangifera indica gum, Pharmaceutical excipients.

IINTRODUCTION

agent.

Robbins has stated, "in spite of the problems which have beset the gums market in recent years, the fact remains that in many cases the gums provide a valuable source of income for many poor smallholders or itinerant labourers, either in very poor countries or in the poorest regions rather than more developed countries as such they are important commodities¹.

For centuries man has made effective use of materials of natural origin in the medical and pharmaceutical field. Today, the whole world is increasingly interested in natural drugs and excipients. Natural materials have advantages over synthetic materials because they are non toxic, less expensive and freely available. Furthermore, they can be modified to obtain tailor made materials for drug delivery systems allowing them to compete with the synthetic products that are commercially available. Many kinds of natural gums are used in the food industry and are regarded as safe for human consumption. It should be noted that many 'old' materials are still popular today after almost a

RGUHS Journal of Pharmaceutical Sciences Received: 24/01/2011, Modified: 01/03/2011, Accepted: 07/03/2011 century of efforts to replace them. It is usual to strike a balance between economics and performance in the face of commercial realities²⁻⁵.

Natural gums obtained from plants have diverse applications in drug delivery as disintegrant, emulsifying, suspending agents and as binders. They have also been found useful in formulating immediate and sustained release preparations³⁻¹⁰.

For centuries, the Mango tree (Scientific name: *Mangifera indica*, Family: Anacardiaceae) has been an integral part of life in India. Each and every part of the tree (bark, leaves, root and kernel seed fruit) serves a certain purpose, for instance, as diuretic, astringent, aphthous stomatitis, diabetes, asthma, diarrhea, urethritis, dysentery, scabies and other parasitic skin diseases¹¹. Literature survey reveals that comprehensive physicochemical characterization and pharmaceutical application of the *Mangifera indica* gum (MIG) as a disintegrating agent in tablet formulation has not been reported yet.

Many patients, especially elderly find it difficult in swallowing tablets, capsules, fluids and thus do not comply with prescription, which results in high incidence of noncompliance oriented research has resulted in bringing out many safer and newer drug delivery systems. Rapidly disintegrating/dissolving tablet is one of such example, for the reason of rapid disintegration or even with saliva. Significance of this drug delivery system includes administration without water, accuracy of dosage, ease of portability, alternative to liquid dosage forms, ideal for paediatric and geriatric patients and rapid onset of action¹².

Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose concentrationhyperglycemia-caused by insulin deficiency, often combined resistance¹⁹. Metformin HCl, an important with insulin drug of biguanide class, is currently available drug for treating hyperglycemia in (Non-Insulin Dependent Diabetes Mellitus (NIDDM); but has been associated with severe and sometimes fatal hypoglycemia and gastric disturbances like nausea, vomiting, heartburn, anorexia and increased appetite after oral therapy. Since these drugs are usually intended to be taken for a long period, patient compliance is also very important²⁰⁻²¹. Metformin, which is slowly and partially absorbed by the gut, is taken in the form of oral tablets of 500 and 850mg, usually at a dose of 2g (maximum of 3g) per day. The absolute bioavailability of a 500mg immediate-release tablet is about 50 to 60%; the half-life is 2 -6h and the maximum plasma concentration is reached after 2.5h, Almost 80-100 % of the drug is excreted unchanged. In the Bioavailability Classification System (BCS), metformin is classified as a class III drug, because of its high water solubility.

The objective of the present study was to isolate MIG from its source and to study physicochemical and phytochemical parameters to ascertain its suitability as a disintegrant for developing fast disintegrating tablets (FDT's) of the selected model drug metformin HCl. The disintegration and swelling properties of FDT were compared with widely used super disintegrant like Crosspovidone®. Metformin HCl, an antidiabetic drug, was selected as the model drug as it was widely used in the treatment of Type–II diabetes.

MATERIALS AND METHODS

Materials:

Metformin HCl, talc, magnesium stearate, aspartame, aerosil were obtained from Zydus Research centre, Ahmedabad, India as gift samples. Mango gum resin was collected from the incised trunk of *Mangifera indica* in Ankola region (Uttar Kannada District) region. All the other solvents, reagents and chemicals used were of either Pharamcopoeial or analytical grade. Different instruments *viz*; Vernier calipers, Monsanto hardness tester, Roche friabilator and disintegration apparatus were supplied by Campbell Electronics, Mumbai. USP XXIII dissolution apparatus-2 was from Tab- Machines, Mumbai, 1601 PC Shimadzu UV Spectrophotometer from Tokyo, Japan and Shimadzu DSC-60, Shimadzu Limited Japan.

Methods:

Extraction of *Mangifera indica* Gum:²²⁻²³

The mango gum resin gum was collected from *Mangifera indica* trees (injured trunk site). It was dried, ground, and passed through sieve no 80. Dried gum (15 g) was stirred in distilled water (300 ml) for 6-8 h at room temperature. The supernatant was obtained by centrifugation. The residue was washed with water and the washings were added to separate supernatant. The procedure was repeated four more times. Finally the supernatant was made up to 500 ml and treated with twice the volume of acetone by continuous stirring. The precipitated material was washed with distilled water and dried at 50-60°C under vacuum. The dried gum was pulverized using a pulverizer and stored in tightly closed container.

Evaluation of Toxicity:

Toxicity studies were carried out according to the method of Knudsen and Curtis²⁴. The animals used in the toxicity studies were sanctioned by the Institute Animal Ethical Committee (Approval No: KLECP/IAEC/45/2010-11). The male albino rats of Wistar strain weighing 160-200 g were divided into different groups comprising of six animals each. The control group received normal 0.5%CMC solution (20ml/kgi.p). The other groups received 500, 1000, 2000, 3000, 4000 and 5000 mg/kg of MIG suspension in normal saline orally. The animals were observed continuously for the behavioral changes for the first 4 hours and then observed for mortality if any for 72h. Since no mortality, no toxic manifestations were observed and behavioural pattern was unaffected. In chronic toxicity studies, 22 animals were used, divided in to two groups, 6 as control and 16 as test animals. In the test group a dose of 500 mg/kg was administered daily for a period of 30 days Body weights were recorded for both the groups at an interval of 10day and at the end of 30 days, hematological and biochemical parameters were studied in both the groups and after 30 days of chronic toxicity study the animals were scarified and subjected to histopathological studies.

Physicochemical characterization of mucilage:²⁵⁻²⁸

The physicochemical properties such as solubility, swelling index, ash values, loss on drying, precompression parameters and microbial load of the MIG were determined according to official Procedures. The following evaluation parameters were carried out as per the procedures described below.

Solubility:

The separated gum was evaluated for solubility in water, acetone, chloroform, methanol, ether and ethanol in accordance with the British Pharmacopoeia specifications.

Determination of swelling index:

Swelling characteristics of the separated MIG powder was studied in different media such as 0.1 N hydrochloric acid, pH 7.4 phosphate buffer and distilled water. The swelling index is the volume in ml occupied by 1 g of drug; including any adhering gum after it has been swollen in an aqueous liquid for 4 h. The swelling index of MIG powder was determined according to the British Pharmacopoeia method. 1g of MIG powder was taken in a 25 ml ground glass stoppered cylinder graduated over a height of 120 to 130 mm in 0.5 divisions. To this 25 ml of respective medium was added and this was shaken vigorously every 10 m for 1 h and then allowed to stand for 24 h. The volume occupied by the MIG powder was measured.

The swelling index was computed using the equation

$$\mathbf{S} = \mathbf{V}\mathbf{2}/\mathbf{V}\mathbf{1}.$$

Where; S = Swelling index

V1 = Volume occupied by the gum prior to hydration

V2 = Volume occupied by the gum after to hydration

The test was carried out in triplicate and the average value of swelling index was recorded

Loss on drying:

As the inherent moisture in MIG powder/excipients may influence the stability of the tablet dosage form containing moisture sensitive drugs, moisture content of the separated mucilage was detected by loss on drying method. The sample (1 g) was heated at 105°C until constant weight in a hot air oven and percentage loss of moisture on drying was calculated using the formula,

LOD (%) = (weight of water in sample/weight

of dry sample)x100.

Total ash:

The total ash was determined by placing 3 g of the ground air-dried material in a crucible, spreading the material in an even layer and igniting it by gradually increasing the temperature to 550°C until it is white, indicating the absence of carbon. The crucible was cooled in a desiccator, weighed and the content of total ash in mg per g of air-dried material was calculated.

Acid Insoluble ash:

Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. To the crucible containing the total ash, 25 ml of hydrochloride acid was added, covered with a watch glass and boiled gently for 5 min. The watch glass was rinsed with 5 ml of hot water this liquid was added to the crucible. The insoluble matter on an ash less filter paper was collected and washed with hot water until the filtrate is neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight. The residue was allowed to cool in a desiccator for 30 min, weighed without delay and the content of acid insoluble ash in mg per g of airdried material was calculated.

Microbial load:

Microbial count for separated MIG powder was performed as outlined in Indian Pharmacopoeia-1996 for total aerobic microbial count using plate count method. The plate count for bacteria and fungi were measured.

pH determination:

This was done by shaking a 1%w/v dispersion of the sample in water for 5 min and the pH determined using a pH meter (Elico, Hyderabad). The data presented here is for triplicate determinations.

Angle of repose:

The static angle of repose, a, was measured according to the fixed funnel and free standing cone method. A funnel was clamped with its tip 2 cm above a graph paper placed on a flat horizontal surface. The powders were carefully poured through the funnel until the apex of the cone thus formed just reached the tip of the funnel. The mean diameters of the base of the powder cones were determined and the tangent of the angle of repose calculated using the equation:

Tan a = 2h/D

The data presented here is for triplicate determinations.

Bulk and Tapped densities:

2 g quantity each of the powder sample was placed in a 10ml measuring cylinder and the volume, $V_{\rm o}$, occupied by each of the samples without tapping was noted. After 100 taps on the table, the occupied volume $V_{\rm 100}$ was read. The bulk and tap densities were calculated as the ratio of weight to volume ($V_{\rm o}$ and $V_{\rm 100}$ respectively). The data presented here is for triplicate determinations.

Hausner's index:

This was calculated as the ratio of tapped density to bulk density of the samples.

Compressibility index:

This was calculated using the equation:

Compressibility = (Tapped density- bulk density)/Tapped density x 100.

Differential Scanning Calorimetry (DSC) Analysis:

Thermal properties of MIG powder were characterized using a Shimadzu DSC-60, Shimadzu Limited Tokyo, Japan. Nitrogen, at the rate of 20 ml/min, was used as purge gas; 2 mg of powdered material were sealed in aluminium pan and heated from 30°C up to 400°C at the rate of 10°C/min, followed by a cooling cycle back to 30°C at the same rate.

Fourier Transform Infra Red (FT-IR) Analysis:

The FT-IR spectrum of the sample was recorded in an IR spectrometer (FT–IR: 8101 M, Shimadzu, Japan), using potassium bromide (KBr) discs prepared from powdered samples mixed with dry KBr in the ratio 1:200. Triplicate measurements were made, and the spectrum with the clearest identifiable peaks was chosen.

Phytochemical Examination:²⁹

Preliminary tests were performed to confirm the nature of gum obtained. The chemical tests that were conducted are: Ruthenium red test, Molisch test, test for reducing sugars and Ninhydrin test.

Characterization of Drug and Excipients

Drug-excipient compatibility studies:

This study has been done to check whether there is any compatibility related problems are associated with drug and the excipients used for the formulation of mouth dissolving tablets. The drug and excipients must be compatible with one another to produce a product that is stable, efficacious, attractive, and easy to administer and safe. If the excipients are new and not been used in formulations containing the active substance, the compatibility studies are of paramount importance. Thermal analysis, TLC, HPLC, FTIR, can be used to investigate and predict any physicochemical interactions between components in a formulation and can therefore be applied to the selection of suitable chemically compatible excipients.

Fourier Transform Infrared (FTIR) Spectroscopy:

FTIR spectra were recorded on samples prepared in potassium bromide (KBr) discs using a IR spectrometer (FT–IR: 8101 M, Shimadzu, Japan), Samples were prepared in KBr disks by means of a hydrostatic press at 6-8 tons pressure. The scanning range was 500 to 4000 cm⁻¹.

Differential Scanning Calorimetry (DSC):

DSC analysis was performed using Shimadzu DSC-60, Shimadzu Limited Japan. A 1:1 ratio of drug and excipient was weighed into aluminum crucible. And sample was analyzed by heating at a scanning rate of 20°C over a temperature range 20°-300°C under nitrogen environment.

Standard Calibration Curve of Metformin Hcl:

Solutions ranging from 2 to 4 μ g/ml were prepared in phosphate buffer (pH 6.8). Absorbance was measured for each solution at λ_{max} of 233 nm, using 1601 PC Shimadzu UV Spectrophotometer. Correlation coefficient was found to be 0.9998 in phosphate buffer.

Formulation of Mouth Dissolving Tablets:

Mouth dissolve tablets of Metformin HCl were prepared by the conventional direct compression technique using MIG powder and compared with different concentrations viz; 2, 4, 6, 8 and 10% (w/w) of *Mangifera indica* gum powder and crosspovidone®. All ingredients were passed through mesh no.60. Required quantity of each was taken for particular formulation and the blend was mixed by tumbling in a polythene bag. The composition of each formulation is given in Table 1.

Evaluation of powder Blend

Pre compression parameters:³⁰

The prepared powder blend was evaluated for various parameters like bulkiness, bulk density, tapped density, angle of repose, compressibility index and Hausner ratio. After evaluation of powder blend the tablets were compressed with Cadmach single punch compression machine using 12mm flat faced punches.

Evaluation of tablets

Post compression parameters:³¹

After tablet compression, all the tablets were evaluated for different parameters as thickness, hardness, friability, uniformity of weight, disintegration time, water absorption ratio, wetting time, drug content. *In vitro* dissolution studies were carried out in USP dissolution test apparatus (Type 2), using simulated intestinal fluid (pH 6.8) (900ml, $37 \pm 0.5^{\circ}$ C) at 50 rpm.

pH of the solution:

The pH of the solution was measured using pH meter, after dissolving the tablet in around 200 ml of water.

Accelerated stability studies:

Stability studies were carried out on optimized formulation as per ICH specifications. The tablets were stored at $25 \pm 2^{\circ}$ C / $60 \pm 5^{\circ}$ RH and $40 \pm 2^{\circ}$ C / $75 \pm 5^{\circ}$ RH for duration

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Table 1: Formulation of Mouth Dissolving Tablets of Metformin HCI										
Ingredients	F1	F2	F3	F4	F5	F6	F 7	F8	F9	F10
(mg/each tablet)										
Metformin HCl	500	500	500	500	500	500	500	500	500	500
MIG*	12	24	36	48	60				_	
Crosspovidone®		_				12	24	36	48	60
Aspartame	6	6	6	6	6	6	6	6	6	6
Magnesium Stearate	12	12	12	12	12	12	12	12	12	12
Talc	3	3	3	3	3	3	3	3	3	3
Aerosil	3	3	3	3	3	3	3	3	3	3
Flavor (Mango)	6	6	6	6	6	6	6	6	6	6
Avicel q.s.to	58	46	34	22	10	58	46	34	22	10
Total weight of tablet	600	600	600	600	600	600	600	600	600	600
		MI	G* Mangii	fera indica	gum					

of three month. After an interval of one month samples were withdrawn and tested for various physical tests and *in vitro* drug release.

RESULTS AND DISCUSSION

Plant products serve as an alternative to synthetic products because of local accessibility, environment friendly nature and lower prices compared to imported synthetic products. Herbs are non-polluting renewable resources for sustainable supplies of cheaper pharmaceutical products. Today, we have a number of plant-based pharmaceutical excipients. A number of researchers have explored the utility of plant-based materials as pharmaceutical excipients. Majority of investigations on natural polymers in drug delivery systems are centered on polysaccharides and proteins, due to their ability to produce a wide range of materials and properties based on their molecular structures.

Gums derived from the plant of *Mangifera indica* was investigated as disintegrating agent for use in moth dissolving tablet formulations containing metformin HCl.

Physicochemical characterization of *Mangifera indica* gum

The average yield of dried gum obtained from *Mangifera indica* tree was 35% w/w. The gum obtained was an off white to cream yellow color powder, and the viscosity of its 1% aqueous dispersion was 600 cP. The powder was slightly soluble in water and practically insoluble in ether, acetone, chloroform, methanol and ethanol.

The swelling characteristic of MIG was studied in different media; 0.1N hydrochloric acid, phosphate buffer (PH 7.4) and water. The swelling was highest in water (20) followed by 0.1N HCl pH (15) and least in phosphate buffer (10). Generally, the results show that MIG has high

swelling index suggesting that the gum may perform well as binder/disintegrant/matrixing agent. The gum is a pH responsive polymer, it is therefore a "smart polymer," and may find application in controlled release dosage formulations. The moisture content of MIG was low (1.5%), suggesting its suitability in formulations containing moisture sensitive drugs. The total ash, water soluble ash and acid insoluble ash value of MIG was found to be 2.23, 1.3 and 0.4% w/w respectively. Ash values reflect the level of adulteration or handling of the drug. The bulk and tapped densities give an insight on the packing and arrangement of the particles and the compaction profile of a material. The compressibility index, Hausner ratio and angle of repose of MIG were 16.33%, 0.15 and 22.35° respectively, implying that the MIG has a good flow with moderate compressibility. The loss on drying, ash value and microbial count were well within official limits. The gum obtained from Mangifera indica tree was subjected to physicochemical characteristics the results of which are summarized in table 2.

Phytochemical screening of *Mangifera indica* gum

Phytochemical tests carried out on MIG confirmed the absence of alkaloids, glycosides and tannins. On treatment of mucilage with ruthenium red, it showed red colour confirming the obtained product as mucilage. A violet ring was formed at the junction of two liquids on reaction with Molisch's reagent indicating the presence of carbohydrates. Mucilage could not reduce Fehling's solution, so the sugars present were non reducing sugars. It reduced Fehling's solution after hydrolysis for 1h with concentrated sulfuric acid under reflux. Mucilage on treating with ninhydrin reagent does not give purple colouration indicating the absence of amino acids. The results of phytochemical screening of MIG are summarized in table 3.

Toxicity study of MIG

To determine the safety level of extracted MIG, acute and chronic toxicity studies were carried out. In acute toxicity study no mortality was observed even at 5000mg/kg of MIG on oral administration and all animals were found to be normal during and at the end of the observation period of three days. Food and water consumption also did not differ significantly and there was no change in general behavior or other physiological activities of the animals in both control and treated groups. To assess the suitability of MIG for the oral delivery we have recorded the body weight profile for the animals during the chronic toxicity studies at regular intervals of 10 days. It was found that the body weight of both control and treatment group and the rate of increase in body weight were comparable. Hence, it could be inferred that chronic administration of the gum might not influence either the food intake or growth. Biochemical and hematological parameters were determined at the end of 30 days of continuous administration of MIG suspension and the biochemical and hematological parameters were found to be comparable to that of normal mice. The results are shown in table 4 and 5 respectively. Histological examination of the main organs like liver, kidney, heart and brain were carried out at the end of 30days of chronic toxicity study. From this study it was revealed that there was no sign of pathological changes in both control and in treatment group.

Characterization of MIG

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) was used to measure the occurrence of exothermal or endothermal changes with increase in temperature. DSC, because of its sensitivity and accuracy, has been extensively used to study the phase transitions of polymers. The thermogram for MIG is shown in Figure1.It shows that the gum has both amorphous and crystalline portions. Glass transition (Tg) temperature occurred at 94°C while a melting peak was observed at about 320°C.

Fourier Transform Infra Red (FT-IR)

The IR spectrum of MIG is shown in Figure 2. The finger print region of the spectrum consists of two characteristic peaks between 700 and 1316 per cm, attributed to the C-O bond stretching. The band at 1604/cm was assigned to the O-H bending of water. There are absorptions (weak) in the 1730 per cm area that indicate carbonyls. The absence of significant aromatic stretches in the 1660-1690/cm region

Table 2: Physicochemical characterization of Mangifera indica gum						
Parameters	Observation					
Solubility	Slightly soluble in water, practically insoluble in alcohol, chloroform and acetone. Forms thick gel in water.					
pH (1% w/v solution)	6.5					
Loss on drying	1.5%					
Ash value	2.23%					
Water soluble ash	1.3%					
Acid insoluble ash	0.4%					
Sulphated ash	1.03%					
Test for foreign matter	Less than 0.1%					
Test for arsenic	Less than 1ppm					
Swelling ratio						
In water	20.0					
In 0.1 N HCl	15.0					
In phosphate Buffer 7.4	10.0					
True density	1.7g/dl					
Bulk density	0.48 g/cc					
Tapped density	0.56 g/cc					
Compressibity index	16.33%					
Hausner ratio	0.15					
Angle of repose	22.35					
State	Amorphous powder					
Odor	No characteristic odor					
Taste	Tasteless					
Color	Off white- cream yellow color					
Total bacterial count						
E.coli	Absent					
Salmonella typhi	Absent					
S. aureus	Absent					
Yield (%)	35					
Viscosity (1%)	600 centipoise					

and the weakness of the stretches, imply that there is a modest amount of peptidic cross linking by amide bond formation. The sharp band at 2939 per cm is characteristic of methyl C-H stretching associated with aromatic rings. The broad band at 3286 cm⁻¹ is due to the hydrogen-bonding that contributes to the complex irrational stretches associated with free inter and intra-molecular bound hydroxyl groups which make up the gross structure of carbohydrates.

Drug Excipient Compatibility Study

Fourier Transform Infrared (FTIR) Spectroscopy

The IR spectral analysis of metformin HCl and the physical

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	Table 3: Phytochemical screening of Mangifera indica gum							
	Tests	Observation						
1.	Test for Carbohydrates(Molisch 's test)	+						
2.	Test for Tannins(Ferric chloride test)	-						
3.	Test for proteins (Ninhydrin test)	-						
4.	Test for alkaloids (Wagner 's test)	-						
5.	Test for glycosides (Keller – Killaini test)	-						
6.	Test for mucilage (Ruthenium red test)	+						
7.	Test for flavonoids (Shinoda test)	-						
8.	Test for reducing sugar (Felhing 's test)	-						
9.	Mounted in 95% alcohol	Transparent angular masses under microscope						
10.	Mounting in the iodine	No blue colored particles (starch absent)						
11.	Test with cupric -tartaric solution	Red precipitate is produced						
12.	Warming with 5M sodium hydroxide	A brown color is produced						
13.	Test for chlorides(silver nitrate test)	-						
14	Test for sulphates (barium chloride test)	<u>.</u>						

Table 4: Results of Biochemical parameters in rats treated with MIG								
Treatment	ALP (U/L)	ACP (U/L)	AST (U/L)	ALT (U/L)	Urea (U/L)	Creatinine (U/L)		
$\frac{\text{Control}}{(0.5\%\text{CMC})^{***}}$	65±4.1	5 [*] 29±4.25	72±2.34	56±1.25	51±2.10	0.4±0.22		
Treatment (MI 500 mg/kg)	$(G)^{****} = 68 \pm 4.33$	8 ^{**} 27±2.02	69±4.10	58±2.87	48±1.65	0.3±0.21		
*Data represents as the mean SD of 6 animals; **Data represents as the mean SD of 16 animals; ***CMC; Carboxy methyl cellulose; **** MIG; <i>Mangifera indica</i> gum								
Tab	le 5: Results o	f Hematologica after treatmen	al changes obs at of MIG for 30	served in ra) days	its during a	ind		
Treatment	RBC	WBC	Hb(g/dl)	Ν	L	E		
	(10 ⁶ /mm ³)	$(10^{3}/mm^{3})$						
Control (0.5% CMC)	$4.3 \pm 0.05^{*}$	7100 ± 0.10	13.58 ± 0.21	8 ± 0.52	85± 0.17	7 0±0.00		
Test(MIG) 500 mg/kg)	$4.1 \pm 0.07^{**}$	6850 ± 0.13	14.12 ± 0.35	12 ± 0.41	90 ± 0.2	$1 1 \pm 0.22$		

*Data represents as the mean SD of 6 animals; **Data represents as the mean SD of 16 animals

mixture of metformin HCl and other excipients are presented in Figure 3 and 4 respectively. Pure metformin HCl spectra showed principal peaks at different wave numbers corresponding to its functional groups, confirming the purity of the drug as per established standards. All the above characteristic peaks appear in the spectra of physical mixture of metformin HCl and other excipients, indicating no modification or interaction between the drug and excipients.

Differential Scanning Calorimetry (DSC)

The DSC analysis (Figure 5) of pure metformin HCl showed a characteristic, sharp endotherm peak at 226°C corresponding to its melting point and indicates the crystalline nature of the drug. The DSC analysis of physical mixture of drug and excipients (figure 6) revealed negligible change in the melting point of metformin HCl in the presence excipients, indicating no modification or interaction between the drug and excipients.

Precompression parameters of powder blend

Since, the flow properties of the powder mixture are important for the uniformity of mass of the tablets, the flow of the powder mixture was analyzed before compression to tablets. Bulk density was found to be between 0.53 to 0.57 g/cc and tapped density between 0.67 to 0.74 g/cc, bulkiness between 1.72 to 1.89, carr's index between 16.9 to 23.7%, Hausner ratio between 1.22 to 1.32 and angle of repose was found to be between 24.6 to 33.6, indicating fair to good flow properties. Results of precompression parameters are shown in Table 6.



Post compression parameters of fast dissolving tablets

Tablets were prepared using direct compression. Tablets were obtained of uniform weight due to uniform die fill, with acceptable weight variation as per Pharmacopoeial specification. Hardness of the all the formulations were measured in kg/cm². The hardness of all formulations was found to be 3-4 kg/cm². Drug content of all the formulations were found to be in the range of 98-101%, which is within acceptable limits. Friability values of all the formulations were within the limit i.e. is less than 1.0% indicated that tablets had a good mechanical resistance. pH of the solution of all the tablets was found to be between 6.3 to 7.5, which suggest that the tablets can be conveniently administered orally and will not cause any discomfort. Results of post compression parameters are shown in Table7.

The separated MIG powder was evaluated for its performance as disintegrant in tablets at various concentrations (2, 4, 6, 8, 10 %w/w) and the optimum concentration found was 6 %. Its performance was compared with crosspovidone at optimum concentration (4%) and it was found better than crosspovidone in tablet

formulations with less disintegration time (51 s) compared to that of crosspovidone (56 s).

Wetting time was used as a parameter to correlate with disintegration time in oral cavity. This is an important criterion for understanding the capacity of disintegrants to swell in presence of little amount of water. Since the dissolution process of a tablet depends upon the wetting followed by disintegration of the tablet, the measurement for the evaluation of dispersible tablets. The wetting time of formulated tablets was found in the range of 38-88 s. In vitro and In vivo dispersion time was 43-65 s for all the formulations. The disintegration times of all the formulations were within official requirements that are less than 180s. Comparison between disintegration time in oral cavity, wetting time and disintegration time (In vitro) for MIG powder formulations are shown in Figure7. Disintegration time in oral cavity was found between 45-68 s for MIG powder. This showed good correlation between disintegration time in oral cavity and wetting time for all formulations.

All designed formulations using MIG powder and Crosspovidone showed rapid dissolution and percent

	Table 6: Results of blend properties of metformin HCI.									
Formula	ation Angl	e of E	Bulk	Tapped	Carr's	Hausner	Bulkiness			
code	repo	$\mathbf{se}(^{\circ})^{*}$ d	lensity	density	index	ratio	$(\mathbf{cc/g})^*$			
		()	$\mathbf{gm/cm}^{3})^{*}$	$(\mathbf{gm/cm}\ 3\)^*$	(%) [*]	$(\mathbf{H} \mathbf{R})^*$				
F1	33.6±	0.02 0	.56±0.02	0.74±0.02	23.7±0.01	1.30±0.04	1.79±0.05			
F2	26.3±	0.01 0	$.54{\pm}0.04$	$0.73 {\pm} 0.03$	22.8 ± 0.01	1.32 ± 0.01	1.75 ± 0.01			
F3	31±0	.04 0	.53±0.01	0.67 ± 0.01	20.8 ± 0.02	1.26 ± 0.05	$1.89 {\pm} 0.02$			
F4	24.6±	0.02 0	$.55 \pm 0.01$	0.70 ± 0.02	19.9 ± 0.04	1.27 ± 0.02	1.82 ± 0.03			
F5	25.0±	0.02 0	.57±0.01	0.74 ± 0.02	23.1 ± 0.01	1.29 ± 0.02	1.75 ± 0.04			
F6	25.1±	0.02 0	$.57 \pm 0.02$	0.71 ± 0.03	19.0 ± 0.01	1.24 ± 0.02	1.75 ± 0.02			
F7	27.9±	0.06 0	.54±0.03	$0.73 {\pm} 0.03$	21.5 ± 0.02	1.35 ± 0.04	$1.72 \pm 0.20.01$			
F8	28.3±	0.04 0	.55±0.03	$0.67 {\pm} 0.02$	16.9 ± 0.03	1.22 ± 0.03	$1.79 {\pm} 0.03$			
*All values are expressed as mean ± SD, n=3.										
Table 7: Results of Post Compression Properties of Metformin dispersible Tablets										
Formu	Thickness	Diameter	Hardne	ss Friability	7 Drug	Weight	pH of			
lation	$(\mathbf{mm})^{*}$	$(\mathbf{mm})^{*}$	(kg/cm ²	²)* (%)***	content	variation	the			
code					(%) ^{**}	$(\mathbf{mg})^{**}$	solution			
F1	4.2 ± 0.01	11.00 ± 0.03	3 2.9±0.16	0.32 ± 0.01	101.91±0.0	$1 204 \pm 0.02$	7.2			
F2	$3.8 {\pm} 0.05$	11.00 ± 0.02	2 3.2±0.14	0.33 ± 0.03	100.12±0.0	4 198±0.04	6.5			
F3	4.1 ± 0.02	11.00 ± 0.02	2 2.8±0.12	0.54 ± 0.06	98.12±0.04	202 ± 0.06	6.4			
F4	$3.9 {\pm} 0.03$	12.00±0.0	1 2.9±0.10	0.55 ± 0.04	99.12±0.01	199 ± 0.01	6.2			
F5	4.1 ± 0.02	11.00 ± 0.04	4 3.1±0.14	0.24 ± 0.05	100.43±0.0	6 199±0.01	7.3			
F6	4.2 ± 0.01	12.00 ± 0.03	3 3.0±0.16	0.21 ± 0.05	101.34±0.0	$5 201 \pm 0.05$	6.6			
F7	4.0 ± 0.01	12.00±0.0	1 3.2±0.16	0.23 ± 0.04	99.45±0.05	198±0.07	7.5			

*All values are expressed as mean ± SE, n=5; **All values are expressed as mean ± SE, n=20; ***All values are expressed as mean ± SE, n=10.

cumulative drug release (% CDR) at the end of 15 min was between 83-98%. The results are shown in Figure 8.

The optimized formulation F3 was kept at real time (25 ± 2 C° C / 60 ± 5 % RH) and accelerated (40 ± 2 C °/75 ±5 % RH) storage conditions for a period of 3 months. After stability test period, tablets were analyzed for drug content, hardness, friability, *in vitro* release and disintegration tests. Stability studies result showed that there was no significant change in hardness, friability, drug content, and dissolution profile of formulation F3. The formulation was stable under accelerated conditions of temperature and humidity.

CONCLUSION

From the present study, it can be concluded that natural super disintegrants like MIG powder showed better disintegrating property than the most widely used synthetic super disintegrants like crosspovidone® in the formulations of FDTs and may be used as disintegrant at the level of 6%w/w in tablet formulations. As primary ingredients are cheap, biocompatible, biodegradable and easy to manufacture. They can be used as superdisintegrants in place of currently marketed synthetic superdisintegrating agents.

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A New Reverse Phase High Performance Liquid Chromatographic Method for Determination of Lornoxicam in Bulk and Tablet Dosage Form

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A rapid, accurate, precise and sensitive RP-HPLC method for the determination of the non-steroidal anti-inflammatory drug lornoxicam (LORN) in bulk and pharmaceutical tablet dosage form has been developed. The analyte is resolved by using a reverse phase phenomenex-luna 5μ C-8 (2) 100Å⁰, 250 × 4.3mm, column at room temperature. The mobile phase used was acetonitrile: 1% triethylamine in methanol (60:40 v/v) at a flow rate of 1.0 mL min⁻¹ on an isocratic HPLC system (shimadzu) consisting of LC 10AT VP liquid pump, SPD 10AVP UV-Visible detector at a wavelength of 289nm. The retention time of LORN is 1.91 min. The linear dynamic range for LORN was 2-24 µg mL⁻¹ with the correlation coefficient was 0.9992. The limits of detection and quantification were 0.3362 and 1.1206 µg mL⁻¹, respectively. The developed method was validated in terms of linearity, accuracy, precision, sensitivity, ruggedness and robustness. The % relative standard deviations of peak areas from all the studies were always less than 2%. The proposed method can be used for routine estimation of LORN in bulk and tablet dosage form.

Keywords: Lornoxicam, Method Validation, RP-HPLC.

INTRODUCTION

Lornoxicam (chlortenoxicam) is 6-chloro-4-hydroxy-2methyl- *N*-2-pyridyl-2H-thieno [2, 3-*e*]-1, 2-thiazine-3carbox- amide-1, 1-dioxide (Fig.1); is a novel non-steroidal anti-inflammatory drug (NSAID) with marked analgesic properties ^[1]. LORN belongs to the chemical class oxicams, which includes lornoxicam, tenoxicam and meloxicam. LORN is a Yellow or slightly yellow powder ^[2]. It works by blocking the action of cyclooxygenase, an enzyme involved in the production of chemicals, including some prostaglandins in the body ^[3]. It is used in musculoskeletal and joint disorders such as osteoarthritis and rheumatoid arthritis; it is also used in the treatment of other painful conditions including postoperative pain ^[4].

Literature survey revealed that various analytical methods such as TLC ^[5], liquid chromatography ^[6], polarographic method ^[7], LC-ESI-MS (liquid chromatography-Electrospray ionization- tandem- mass spectrometric) ^[8], spectrophotometric method ^[9, 10, 11, 12], stability-indicating HPLC ^[13] and RP-HPLC method ^[14, 15] are used for estimation of LORN from its formulations individually and in combination with other drugs.

The proposed RP-HPLC method has an advantage over reported RP-HPLC method, that the total run time was less than four minutes, more precise and more accurate. Hence

RGUHS Journal of Pharmaceutical Sciences Received: 24/01/2011, Modified: 11/02/2011, Accepted: 17/02/2011 the developed chromatographic method can be used for routine analysis of LORN in bulk and pharmaceutical tablet dosage form. The method was validated as per ICH guidelines^[16].

MATERIALS AND METHODS

Instrumentation

The Shimadzu HPLC system consisting of gradient pump (LC-10AT VP pump), mixer (SUS vp Assy (new)), rheodyne injector (20 μ L), UV-VIS dual wavelength detector SPD - 10A VP) and Hamilton syringe (all from Shimadzu, Kyoto Japan) was used. The separations were achieved on a Phenomenex - Luna 5 μ C-8(2) 100 A⁰, 250X4.3mm, column with UV detection at 289 nm. Analytical weighing balance (Shimadzu AUX 200) was used for weighing, sonicator (SONICA 2200MH), vaccum pump (model XI 5522050 of Millipore), Millipore filtration kit for solvents and sample filtration were used throughout the experiment. The Spinchrom CFR software-single channel was used for acquisition, evaluation and storage of chromatographic data.

LORN was obtained gift sample from M/s. Micro Labs, Hosur. HPLC grade acetonitrile (ACN) and AR grade triethylamine (TEA) were obtained from Spectrochem Pvt.Ltd, Mumbai, (India). HPLC grade methanol (MeOH) was obtained from CDH (P) Ltd. Sarigam, (India).

Preparation of mobile phase

A solution of 1% triethylamine prepared in HPLC grade

methanol. HPLC experiments were carried out using binary pump. In one solvent reservoir acetonitrile and in another 1% triethylamine in methanol was taken.

Preparation of standard stock solutions

A working standard of LORN (1000 μ g mL⁻¹) was prepared by dissolving 25 mg in 25 ml volumetric flask with acetonitrile. Further 4 ml of the above stock solution was transferred into a 50 ml volumetric flask and diluted up to the mark with mobile phase to obtain 80 μ g mL⁻¹. Standard calibration solutions of range 2–24 μ g mL⁻¹ for assessment of linearity were prepared from this stock solution by dilution with mobile phase.

Preparation of stock solutions for the commercially obtained tablets

20 tablets each containing 8 mg of LORN were taken and crushed to get fine powder. Then weight equivalent to 25 mg of LORN was transferred into a 25 ml volumetric flask. Add about 20 ml of acetonitrile and sonicated for 5 min. Diluted up to volume with acetonitrile and mixed well (1000 μ g mL⁻¹). Further dilutions were made with mobile phase to get a final concentration of 80 μ g mL⁻¹.

Chromatography

The mobile phase acetonitrile: 1% triethylamine in methanol (60:40 v/v) was selected, because it was found that it ideally resolve the peak with retention time (RT) 1.910 \pm 0.02 min and the same is shown in Fig. 2. Wavelength was selected by scanning standard drug over a wide range of wavelength 200nm to 400nm. The component show reasonably good response at 289 nm. The 20 µl sample was injected and the total run time was 4 min.

Calibration

Aliquots of working standard stock solution of LORN were taken in different standard volumetric flasks and diluted with mobile phase to obtain the final concentrations in the range 2-24 µg mL⁻¹, 20 µl of each solutions were injected into the chromatograph. The evaluation of LORN was performed with UV detector at 289 nm. Peak areas were recorded for all the chromatograms. Calibration curve was constructed by plotting peak areas against the amount of drug in µg mL⁻¹ and the linear relationship was evaluated by calculation of regression line by the method of least squares.

RESULTS AND DISCUSSION

Linearity

To evaluate linearity of the proposed method, different concentrations of the analytes in the range of 2-24 $\mu g~m L^{\rm -1}$

of LORN were analyzed and area plotted graphically as a function of analyte concentration. The results obtained show that the current method is linear for the analytes in the range specified above with a correlation coefficient of 0.9992. (Table 1)

Precision

Precision was determined on an intra-day as well as interday basis and is expressed as the % relative standard deviations (% RSD) of the concentrations found in the spiked samples. Intra-day and Inter-day %RSD for LORN were found between the range of 0.65-1.02 and 0.30-1.11% respectively.

Accuracy

The recovery assessment was performed by analysing samples spiked with known amounts standard LORN. Accuracy was determined at three different levels 50%, 100% and 150% of the target concentration. Results have shown that the mean recovery of the assay is within 99 \pm 1.0%. These experiments were assessed on 3 different days by the same analyst.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) of the developed method were calculated according to ICH guidelines where the approach is based on the signal-to-noise ratio. Chromatogram signals obtained with known low concentrations of analyte was compared with the signal of blank sample. A signal-to-noise ratio 3:1 and 10:1 was considered for calculating LOD and LOQ respectively. The values of LOD and LOQ were 0.3362 and 1.1206 μ g mL⁻¹ respectively.

System suitability

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (*RT*), number of theoretical plates (N) and peak asymmetry (AS) were 1.9±0.02, 4516 and 1.313 respectively for the

Table 1: Results of statistical parameters						
Statistical parameters	Values					
Wavelength(nm)	289					
Linearity ($\mu g mL - 1$)	2-24					
Correlation coefficient(r 2)	0.9992					
Intercept	0.2428					
Slope	0.3918					

evaluated six replicate injections of the drug at a concentration of 8 μ g mL⁻¹. The values were within acceptable limits.

Robustness

The robustness of the method was evaluated by deliberate variation in the method parameters, such as flow rate, mobile phase ratio and wavelength. The change in the chromatographic results was monitored by varying these parameters, and it was found that there was little or no change in the retention time of peaks. However, the peak shapes were quite good at the different mobile phase ratio values. There was no significant change in the chromatographic results with alteration in flow rate and wavelength of measurements.

Application of the developed and validated method to the quantitative determination of LORN in tablets

The validated method was successfully applied to the

Table 2: Analysis of formulation									
Tablet	Label	Amount	% Label	%RSD					
	claim (mg)	Found*	Claim						
Fulactive	8	8.072	100.9	0.65					
Lornica	8	8.014	100.1	0.78					
	* avera	ge of three read	ings						





quantitative determination of LORN in tablet having a content of 8 mg per tablet. The LORN content in the tablet was found to be equal to a mean value of 100.5% (RSD 0.71%) of the label claim. (Table.2)

CONCLUSION

The proposed RP-HPLC method developed is rapid, accurate, precise, and specific for the determination of LORN. The method involves simple sample preparation and a short run time of 4 min. Hence this method can be reliably adopted for routine quality control analysis of LORN in bulk and in its tablet dosage form without any interference of excipients.

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Assessment and Evaluation of Drug Information Services provided by the Clinical Pharmcists in a rural tertiary care Teaching Hospital of South India

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Clinical pharmacists play an important role in providing drug information. Provision of drug information helps in promoting safe and effective drug usage in a specific patient situation. The objective of this study was to assess the drug information services provided by the clinical pharmacists. It was a retrospective, prospective and self administered questionnaire study conducted in a clinical pharmacy department for a period of one year. Eighty one queries were received, 47 (58.02%) from general medicine, 23 (28.39%) from pediatrics, 02 (2.46%) from surgery, 01 from OBG and 08 (11.11%) from pharmacists. Mode of queries received were 47 (58.02%) by direct access, 24 (29.60%) during ward rounds. The time of reply for queries immediately were 29 (35.80%), 36 (44.44%) within a day, 16 (19.75%) within 2 to 4 days for providing drug information. Most frequently used resource was MICROMEDEX health care series. Drug information services helped health care professionals in better care of the patient, safe and effective drug usage and updating their knowledge.

Keywords: Drug Information Services, Clinical pharmacist, MICROMEDEX, DIC

INTRODUCTION

The provision of accurate and timely drug information to health care professionals is an important mechanism to promote safe and effective drug therapy to the patients, but such a provision of providing drug information is lacking in India.⁽¹⁾ It has already been reported that India is a country with significant drug use problems. Irrational and unnecessary prescribing is common and antibiotic resistance is widespread. 'Besides poor drug regulation, lack of independent unbiased drug information for doctors and other health care professionals is the main contributing reason for irrational drug use in the country.⁽²⁾ at present drug information to health care professionals is provided by the manufacturer and medical representatives.

The term 'drug information' was coined in the early sixties and the first drug information center (DIC) was opened at the University of Kentucky Medical Center. ⁽¹⁾ Drug information (DI) is defined as the knowledge of facts acquired through reading, study or practical experience, concerning any chemical substance intended for use in diagnosis, prevention or treatment of disease. It covers all types of information provided, including subjective and objective information, as well as information gathered by scientific observation or practical experience.⁽⁵⁾

Drug information service (DIS) refers to service that

RGUHS Journal of Pharmaceutical Sciences Received: 24/01/2011, Modified: 08/03/2011, Accepted: 14/03/2011 encompasses the activities of specially trained individuals to provide accurate, unbiased, factual information, primarily in response to patient-oriented problems occurred from the healthcare team.^(6,7)</sup>

Drug information center (DIC) refers to the specialized facility that provides drug information to those who need it.

Drug information pharmacist is a person who has completed a course of training in drug information such as the advance course established by the SHPA Committee of Specialty Practice in Drug Information, or equivalent, and specializes in the provision of drug information.⁽³⁾

Provision of drug information to physicians and nurses was identified as an excellent responsibility for the developing clinical pharmacist role.⁽⁴⁾

Drug information from commercial sources is very often biased and hence noncommercial, independent, unbiased source of drug information is important. Drug information centers are established in many hospitals and National Human Rights Commission recommended the establishment of such centers in every hospital.⁽²⁾

In 1962, the first drug information center was initialized at the University of Kentucky Medical Center. The Karnataka State Pharmacy Council established its Drug Information Center in August 1997, there onwards actively took role to provide drug information services to health care providers and patients.

In India many colleges and hospitals have started DIS through clinical pharmacy department. There is a good

response from the physicians for the services provided by the clinical pharmacy department, after one and half decade of introduction of clinical pharmacy services in India. In spite of this, clinical pharmacy education faces many challenges before it can transform the pharmaceutical care practice in India from a product-oriented approach to patient-oriented care.

A few institutions are actively involved in establishing the clinical pharmacy department and providing drug information services in South India. The successful establishment of clinical pharmacy department motivated other institutes to establish the same services in their hospitals.

The drug information provided is very well accepted by doctors, which helps in rationalizing of drug use and better patient care. This study aimed at assessing and evaluating the drug information services provided by the clinical pharmacy department.

METHODS

Study design: Retrospective, prospective and self administered questionnaire study.

Study site: Clinical Pharmacy Department, Adichunchanagiri Hospital and Research Centre, Balagangadaranatha Nagara (B G Nagara), Karnataka, India.

Study materials: Questionnaire, drug information documentation form.

Study procedure: Adichunchanagiri Hospital and Research Centre is a part of Sri Adichunchanagiri Mutt situated in B G Nagara. It is a 750 bedded tertiary care teaching hospital with 15 medical departments. Established in the year 2008, Clinical Pharmacy department is an integral part of the hospital which caters clinical pharmacy services to the health care professionals and provides drug information as a part of the clinical pharmacy activities. The centre is well equipped with well trained staff and a library consisting of textbooks, National and International journals, computers and internet facilities along with electronic database such as MICROMEDEX for provision of various services. The center is managed by 5 well qualified faculty members and 20 postgraduate (Master in Pharmacy) and 17 Pharm D (Post Baccalaureate) students respectively. The drug information service is provided between 9 AM to 5 PM on all days except Sunday and government holidays.

Drug information can be accessed by the mode of telephone, direct access and during ward round participation. The drug information queries were evaluated by the staff before providing replies and answered according to modified systemic approach. The drug information queries provided are documented in a suitably designed drug information documentation form and maintained in a documentation file.

The study was carried in two steps which involves both retrospective and prospective studies including self administered feedback questionnaire. The drug information queries which were received during a period of one year i.e. from November 2009 to October 2010 were evaluated retrospectively, screened and analyzed. Data were collected in the suitable tabular forms. The evaluation was done depending on the parameters like professional status of the enquirer, specialty of practice, mode of receipt of query, purpose of the enquiry, time frame to reply, category of the questions and references used.

The feedback questionnaire was developed by the clinical pharmacy department and also included doctors. A self administered feedback questionnaire was designed in such a way that it included information about the name of doctors, qualification, name of the departments and designation. Questions to evaluate the view of doctors regarding the drug information center and drug information queries provided by the clinical pharmacists were also included. Suggestions to improve the services of clinical pharmacy department were also collected by providing the provision in the questionnaire.

RESULTS

A total number of 81 queries were received for a period of one year with an average of 7 (8.6%) queries per month. Queries received from different departments were as follows, general medicine department 47 (58.02%), pediatrics 23 (28.39%), surgery 02 (2.46%), OBG 1 and pharmacists 08 (11.11%). Medical post graduate students had more queries than clinicians [47 (58.02%) and 23 (28.39%) respectively];interns had 3 (3.70%) gueries and pharmacists 08 (9.87%). Queries were in mode of direct access 47 (58.02%) and during ward rounds 24 (29.60%). The queries were also received by telephone 10 (12.34%). The purpose of the queries received for update of knowledge and improvement of education or academics in the institution were 59 (72.83%), for the better patient care 22 (27.16%). The time of reply for queries, required immediately was 29 (35.80%), within a day 36 (44.44%) and within 2-4 days 16 (19.75%) queries were answered. Printed literature information provided were 46 (56.79%), verbal and written replies were 35 (43.20%). The categories of the queries received were focused on-35 on dosage and administration, 29 on drug therapy and contraindications, 20 on indications, 23 on adverse drug reaction, 13 on poisoning, 7 on

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Table1 : Categoriz	ation of the drug information qu	ıeries. (n=81)
Categorization of queries	Number of queries	Percentage of queries
Specialty		
Medicine	47	58.02
Pediatrics	23	28.39
Pharmacists	08	09.80
Others	03	03.70
Status of the enquirer		
Clinicians	23	28.39
Post graduate students	47	58.02
Interns	03	03.70
Pharmacists	08	09.87
Others	00	00.00
Mode of request		
Ward rounds	24	31.17
Direct access	47	57.14
Telephone	10	11.69
Purpose of query		
Update of knowledge	57	71.43
Better patient care	22	25.97
Education/academics	02	02.60
Time frame to reply		
Immediately	29	35.07
Within a day	36	45.45
Within 2 - 4 days	16	19.48
Mode of reply		
Verbal	26	29.87
Verbal and written	09	11.69
Printed literature	46	58.44
Type of query		
Adverse drug reaction	23	29.87
Drug therapy	20	25.97
Dosage / administration	37	42.86
Cost/ availability	00	00.00
Others	01	01.30

pharmacokinetics and dynamics, 6 on interactions, 2 on availability and cost, 3 on efficacy and 9 other queries like brand names and manufacturers (Table no.1). The resources used were Text books and MICROMEDEX for answering of 74 queries. Internet and other facilities were also used for 29 queries. The frequently used resources are listed in table no. 2.

A total of 120 feedback questionnaires were distributed in 7 departments of the hospital where it contains 30 Doctors in

general medicine, 20 in pediatrics, 20 in surgery, 20 in anesthesia, 25 in OBG, 10 in dermatology, and 20 in orthopedics. Enough time of one week was provided to the doctors to reply for the same. Among 120 feedback questionnaires 102 questionnaires were returned. Among 102, 22 from OBG, 18 from medicine, 17 from surgery, 11 each from orthopedics and anesthesia, 7 from pediatrics and 4 from dermatology were received. Among the respondents 41 were postgraduates, 22 interns and 27 clinicians, 72 (80%) of the respondents knew about the DIC which was

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Table 2: Frequency of resources used for provision of queries			
Sl. No	References	Frequency of Usage	
1.	Micromedex Health Care Series.(Database)	38	
2.	Pharmacotherapy hand book. J.T. Dipiro. 6 th Edition.	01	
3.	AHFS Drug Information 2001.	08	
4.	a. IDR, b. ADR and c. CIMS	02	
5.	Martindale The Complete Drug reference. 32 nd edition.	13	
6.	Text book of toxicology. VV Pillay	01	
7.	WHO Guidelines for treatment of Malaria. 2nd ed	01	
8.	Text book of Pharmacology. KD Tripathi	01	
9.	Elements of Pharmacology .TP Gandhi	01	
10.	Applied Therapeutics, the clinical use of drugs. Koda – Kimble	01	
11.	Antibiotics and chemotherapy 8th ed. Roger Daniel	01	
12.	Drug information hand book	04	
13.	Clinical Pharmacy and Therapeutics. Roger walker	03	
14.	Indian pediatrics. Vol. 45	01	
15.	Pharmaceutical Press journal	01	
16.	Poison Index (Manipal)	06	
17.	Text book of Therapeutics, drug and disease management, 7 th ed. Herfindal	01	
18.	Mosby 's Medical Drug Reference. 1999 - 2000	01	
19.	Internet	18	
20.	Others	04	

established in the hospital and 42 (46.6%) of them knew about the services provided by the Clinical Pharmacy department, only 26 (28.8%) of the respondents utilized the DIC, and 22 (24.4%) were aware of the online DIC existing in the hospital.

DISCUSSION

Among the 81 queries received during the study period, a great number were from general medicine department. The reason may be that the faculty and the students from Clinical Pharmacy department were attending the ward rounds for longer durations and also the usage of great number of drugs in general medicine department that becomes necessary for the need of unbiased drug information. Medical post graduate students had more queries than the clinicians, interns and pharmacists which show that they are more oriented towards updating of their knowledge regarding the drugs. More number of queries were received by direct access and ward rounds which shows that the DIC is closely associated with all the health care professionals and DIC is easily feasible from all the wards in the hospital and clinicians are accepting the services provided by the DIC and also this may be because of availability of clinical pharmacist at the time of prescribing or personal contacts.

Table 3: Feedback details on drug information services from doctors. (n=90)				
Departments		Obstetrics and Gynecology	22	
		Medicine	18	
		Pediatrics	07	
		Dermatology	04	
		Orthopedics	11	
		Surgery	17	
		Anesthesia	11	
đ	Physician	Professors	05	
		Associate professors	04	
tio		Assistant professors	17	
osi		Lecturers	01	
Ч		Post graduates	10	
		Interns	22	

Majority of the queries were related to dosage and administration of drugs and contraindications. This may be because of updating the drug monographs, non availability of information, not studied in their curriculum, advanced change of the drug response, new informations added to the drug monographs as and when it is required.

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	Table 3a: Feedback Questionnaires and responses		
S1. N	o Questions	Yes	No
1	Are you aware of clinical pharmacy department established in our hospital?	72	18
2	Are you aware of what services clinical pharmacy department is providing?	42	48
	If yes		
	Drug information	38	
	Adverse drug reaction detection/monitoring/evaluation/reporting	29	
	Patient medication counseling	14	
	Medication chart review	13	
	Any other drug related problems	10	
3	Have you utilized drug information services any time?	26	64
	If yes		
	Have you received the answer in time?	25	1
	Have you received the appropriate answer?	22	0
	If no, the reason was	0	
	Out dated	0	
	Two extensive	0	
	Not relevant	0	
	Others (specify)	0	
4	Do you aware of online drug information exist in our hospital?	22	68
	If yes		
	Have you utilized the facility?	06	13
5	Do you think that the drug information service provided by the department is		
	useful and helps in providing better patient care?	61	29
6	How do you rate the existing drug information system in our hospital?		
	Good	14	1
	Satisfactory	25	5
	Needs improvement	5	1

Secondary sources like electronic database and tertiary sources such as text books were most commonly used resources for answering the queries. The ease of extraction of information from MICROMEDEX and text books explains the extent of the use of these resources since recent and relevant information makes these equally important for search strategy. Websites were also used to answer the queries in less extent compared to both of the above.

Evaluation of the feedback questionnaire showed that most of the enquirers appreciated the quality of the services provided by the drug information center. However, many of the respondents suggested for the need of improvement in the DIC services that were provided.

Among the respondents 48 were not aware of the services provided by the clinical pharmacy department, and 64 have not utilized the services of the clinical pharmacy department. This may be due to the reasons like change of interns, new PGs and new faculties. 61 (67.7%) members thought that the drug information provided by the DIC was useful and helped in better patient care. 51 (56.6%) respondents thought that the DIC needs an improvement in its service, and 14 (15.5%) of them rated the service provided was good and 25 (27.7%) rated as satisfactory.

Some of the suggestions provided by clinicians are as follows:

« To create awareness about the clinical pharmacy department and its services,

« To build good interaction along with postgraduates and interns,

 \ll To allot an in-charge pharmacist for each ward (including ICU & ICCU),

« To provide handouts of new drugs and updated information regarding adverse drug reactions and also to

extend the drug information services round the clock (if possible online services)

The feedback questionnaires which were received were evaluated critically and 12 of them were rejected since they did not give proper information about the respondents. The reasons for rejecting were as follows, all 12 rejected feedback questionnaires were not signed, and along with that 2 did not give the information of the designation, and 1 contained no name.

CONCLUSION

On the whole, the drug information services provided by the clinical pharmacy department of the Sri Adichunchanagiri Hospital and research centre, B G Nagara, caters to the need of health care professionals towards better patient care. Besides, it is essential to create awareness of these services among physicians, pharmacists, nurses and consumers so that they should come forward to take advantage of these services.

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Pharmacodynamic interaction of Calcium channel Blockers with garlic during Isoproterenol induced Myocardial Damage in Rat

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Aim of study: Although the beneficial role of adding traditional healer garlic (*Allium sativum*) to the conventional cardiovascular regimen has been well demonstrated, the interaction between garlic and calcium channel blockers in animals remain unexplored. We investigated the pharmacodynamic interaction of nifedipine, verapamil and diltiazem with garlic homogenate (GH), on isoproterenol (ISO) induced myocardial damage in rat.

Materials and Methods: Albino rats were treated orally with GH (250 and 500 mg/kg) for 30 days, whereas, calcium channel blockers (CCBs) were administered for seven days. Myocardial damage was induced by ISO (150 mg/kg, s.c.) for two consecutive days. Biochemical, electrocardiographic, haemodynamic and histopathological parameters were studied.

Results: Administration of GH resulted in decrease in LDH and CK-MB activities in serum and increase in heart tissue
 homogenate (HTH) compared to ISO control. The SOD and Catalase activities were found to be increased in all treated
 groups compared to ISO control. The QRS duration, QT segment and RR intervals were normalized by GH and CCBs
 treatments. Histological scores confirm the biochemical and electrocardiographic findings.

Conclusion: Among the various combinations, GH 250 mg/kg with diltiazem was substantially more protective than GH with nifedipine/verapamil thereby providing strength to myocardium during stress.

Keywords : Calcium channel blockers; Garlic; Interaction; Isoproterenol

INTRODUCTION

The use of herbal medicines among patients receiving cardiovascular pharmacotherapy is ubiquitous globally. Providing accurate and clinically relevant advice to patients regarding the possibility of herb-drug interactions is a challenge for the healthcare professionals. Simultaneous administration of herbs and drugs may mimic, magnify or oppose the pharmacological effects of each other¹. In view of the increasing use of herbal remedies by the general public and subsequent interest by the physicians, it is imperative to promote credible research on the safety of herbal products including the possibility of interactions with concurrent cardiovascular pharmacotherapy.

Garlic (*Allium sativum, family: Lilliaceae*) is one of the herbs that are widely believed to hold promise as therapeutically effective medicament for cardiovascular diseases. Epidemiologic studies show an inverse correlation between garlic consumption and progression of cardiovascular diseases². Garlic and its preparations have been widely recognized as agents for prevention and treatment of

RGUHS Journal of Pharmaceutical Sciences Received: 27/01/2011, Modified: 20/02/2011, Accepted: 27/02/2011 cardiovascular and other metabolic diseases such as atherosclerosis, arrhythmia, hyperlipidemia, thrombosis, hypertension and diabetes³.

Earlier reports on the drug interaction studies of garlic with calcium channel blockers (CCBs) indicate that it produces concentration dependent synergistic effect due to its own calcium channel blocking effect⁴. However, no scientific observations are available regarding the interaction of garlic with CCBs during myocardial damage. Hence, the present investigation was designed to explore interaction between CCBs namely nifedipine (NE), verapamil (VL) and diltiazem (DM) and different doses of garlic in isoproterenol induced damage to myocardium in rats

MATERIALS AND METHODS

Experimental animals: Laboratory bred Wistar albino rats (200-250 g) of either sex were housed at $25^\circ \pm 5^\circ$ C in a wellventilated animal house under 12:12 h light dark cycle. The rats had free access to standard rat chow (Amrut Laboratory Animal feed, Maharashtra, India) and water *ad libitum*. The animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The Institutional ethical committee approved the experimental protocol (KCP/IAEC-19/2008-9).

Chemicals: All chemicals used were of analytical grade and purchased from standard companies. Biochemical kits like lactate dehydrogenase (LDH) and creatinine kinase-MB (CK-MB) were procured from Crest Biosystems (Goa, India). Nifedipine, verapamil and diltiazem were purchased from the retail pharmacy outlet. Uniformity of brand was maintained throughout study.

Preparation of garlic homogenate and selection of doses of CCBs

Garlic (*Allium sativum, family: Lilliaceae*) bulbs were purchased from the local market. The cloves were peeled, sliced and ground into a paste and suspended in distilled water. Two different concentrations of the garlic homogenate (GH) were prepared, 0.1 and 0.2 gm/ml, corresponding to 250 mg and 500 mg/kg body weight of animal⁵. GH was administered within 30 min of preparation. The oral doses of calcium channel blockers were selected based on earlier reports; nifedipine 6 mg/kg⁶; diltiazem 30 mg/kg⁷. and verapamil 30 mg/kg⁸.

Experimental Protocol

Adult albino rats weighing 200-250 g were divided into following thirteen groups consisting of eight animals each: group I: distilled water (1 ml/kg, p.o. for 30 days); groups II: received Isoproterenol (150 mg/kg, s.c.); group III and IV garlic homogenate (GH) 250 and 500 mg/kg respectively for 30 days orally; group V: Nifedipine (NE) 6 mg/kg for seven days, p.o.; groups VI and VII GH 250 and 500 mg/kg respectively 30 days po + Nifedipine during the last seven days; group VIII were given verapamil (VL) 5 mg/kg, p.o. for seven days; group IX and X received GH 250 and 500 mg/kg respectively for 30 days po + VL during the last seven days; group XI diltiazem (DM) 15 mg/kg for seven days po; group XII and XIII GH 250 and 500 mg/kg respectively for 30 days po + DM during the last seven days. At the end of treatment period, animals of all groups excluding group I were administered ISO (150 mg/kg s.c) for two consecutive days. Blood was withdrawn from retro-orbital vein 48 hrs after the first dose of ISO under ether anesthesia and serum was separated by centrifugation for lactate dehydrogenase (LDH) and creatine phosphokinase-MB (CK-MB) measurement. The ECG changes were recorded under appropriate conditions. The heart was immediately isolated from each animal under ketamine (70 mg/kg, i.p) and xylazine (10 mg/kg, i.p) anesthesia. In each group consisting of eight animals, four excised hearts were homogenized to prepare heart tissue homogenate (HTH) using sucrose (0.25 M)⁹. The activity of LDH, CK-MB, superoxide dismutase (SOD, by nitro blue tetrazolium method)¹⁰ and catalase (hydrogen peroxide method)¹¹ were measured in HTH. Microscopic slides of myocardium were prepared for histopathological studies from the hearts of remaining four animals. The myocardial damage was determined by giving scores depending on the intensity of damage as follows¹²; no changes – score 00; mild – score 01 (focal myocytes damage or small multifocal degeneration with slight degree of inflammatory process); moderate – score 02 (extensive myofibrillar degeneration and/or diffuse inflammatory process); marked – score 03 (necrosis with diffuse inflammatory process).

Statistical analysis

Results are expressed as mean SEM. Statistical significance was assessed using One-way Analysis of variance (ANOVA) followed by Tukey multiple comparison tests. p<0.05 was considered significant.

RESULTS

Effect on biochemical parameters, antioxidants and histological scores in rats

Administration of two doses of ISO resulted in significant fall in LDH, CK-MB, SOD and Catalase activities in heart tissue homogenate (HTH) and increase in LDH and CK-MB in serum compared to normal control. Pretreatment of animals with GH 250 and 500 mg/kg showed significant rise in LDH, CK-MB, SOD and Catalase activities in HTH compared to ISO control. Incorporation of CCBs during chronic treatment of GH was found to further elevate (P <0.001) the LDH, CK-MB, SOD and Catalase activities in HTH and depletes the LDH and CK-MB activities in serum (Table no. 1). The rises in antioxidant and LDH and CK-MB were found to be more in groups treated with GH 250 mg/kg along with diltialzem. The histological scores were significantly lowered in diltiazem, VL, diltiazem+GH, VL+GH, NE+GH as well as GH alone groups compared to ISO control (Table 1). The myocardial integrity was disturbed by administration of ISO for two days (figure 2a) which were found to be prevented by prophylactic administration of garlic either alone or along with diltiazem/nifedipine/verapamil (figure 2b-2f). Administration of nifedipine was found to cause worsening of damages caused by ISO that was reverted to normalcy in animals prophylactically treated with GH 250 mg/kg (2e).

Effect on electrocardiographic parameters and heamodynamic findings

Pretreatment of animals with GH alone or with nifedipine/verapamil/diltiazem were found to keep the integrity of myocardium intact at times of ISO induced stress. The ISO induced increase in HW/BW ratio, QRS duration, QT segment and RR intervals were significantly reduced by administration of GH alone or along with CCBs. Diltiazem and verapamil were found to be effective in bringing back the normalcy to the myocardium. However, Diltiazem restores more accurately QRS duration and QT segment than RR intervals, which upon concurrent administration with GH 250 mg/kg, was also found to be intact. The ISO induced tachycardia was also found to fall significantly in GH and/or CCBs treated groups (Table 2).

DISCUSSION

The purpose of the present research was to explore the role of different doses of garlic homogenate (GH) and its possible interaction with CCBs during ISO induced myocardial damage in rat. The results of the present study demonstrated the stronger ameliorating effect low dose of GH (250 mg/kg) than the high dose of GH (500 mg/kg) during ISO induced myocardium damage. Moreover, present study showed synergistic cardioprotective effect when CCBs were combined with GH.

Three different classes of CCBs were opted in the study based on differences in their pharmacological actions. The dihydropyridines are more potent vasodilators than verapamil, which is more potent than diltiazem. Nifedipine, a dihydropyridine has a good vasodilator effect with little or no cardiac depressant actions. They cause lowering of arteriolar resistance and blood pressure and hence improvement in contractility as well as segmental ventricular function can be achieved. Verapamil, a phenylalkylamine, known to be a potent cardiac depressant with very little vasodilation. Diltiazem (benzothiazepine) has moderate cardiac depressant action with fall in mean arterial blood pressure. It has limited ability to induce marked peripheral vasodilation and reflex tachycardia¹³.

Prophylactic administration of GH with or without nifedipine, verapmil and diltiazem causes substantial protection from toxic manifestation of ISO. This is demonstrated by alleviation of specific stages of myocardial necrosis such as interstitial space, infiltration of leucocyte, nuclear duplication and myocyte size¹⁴.

It is well established that the biological markers such as endogenous enzyme are organ specific and leak from the damaged organ during necrosis¹⁵. Chronic administration of low and high doses of GH keeps the myocardium intact preventing the damage to cardiac cells resulting in elevated activities of LDH and CK-MB in HTH and depleted activities in serum. Oxygen free radicals (OFRs) are involved in the pathophysiology of wide range of disease conditions including ischemic heart diseases, resulting usually from deficient endogenous antioxidant defenses. It can be emphasized that many of ischemic heart disease occurs due to imbalance between oxidants and antioxidant defenses. Potential antioxidant therapy should include the supplementation of endogenous antioxidants with natural antioxidants or augmentation of endogenous antioxidant synthesis such as superoxide dismutase, catalase, etc¹⁶. Among number of OFRs associated with myocardial contractile and rhythmic disturbances, contribution of superoxide to myocardial damage is believed to be the highest and this radical is combated by elevated activities of endogenous antioxidant enzyme - the superoxide dismutase (SOD)¹⁷. In addition to this, measurement of Catalase activity was carried out as elevation in SOD dismutes superoxide but results in accumulation of H₂O₂ which could further precipitate the MI¹⁸. Chronic administration of GH (250 and 500 mg/kg) alone or along with NE/VL/DM produced elevation in SOD and Catalase activities indicating augmented synthesis of endogenous antioxidants during garlic treatment. The maximum activity was seen with GH 250 mg/kg in presence of DM indicating combined antioxidant potential.

One of our important findings was safety and increased efficacy of nifedipine when used with garlic. Nifedipine alone was found to cause worsening of ischemic damage induced by ISO. The worsening conditions may have resulted from excessive hypotension and decreased coronary perfusion, selective coronary vasodilation in non ischemic regions of the myocardium in a setting where vessels perfusing ischemic regions were already maximally dilated (*i.e.*, coronary steal), or an increase in oxygen demand owing to increased sympathetic tone and excessive tachycardia. The combined therapy of nifedipine and garlic was found to provide protection to the myocardial membrane thereby preventing its damage and release of biological markers in the serum. The inflammation and necrosis were also prevented when nifedipine was added during chronic garlic therapy.

Our studies are in line with the earlier view that nifedipine had a detrimental effect on mortality due to myocardial infarction. It was also known that diltiazem and verapamil might reduce the incidence of reinfarction in patients. However, beta-blockers remain the preferred drug for MI¹⁹. Concurrent administration of moderate dose of garlic (250 mg/kg) and nifedipine found to prevent the aggravation of damages and keeps the integrity of myocardium intact. Similarly, combined therapy of garlic with verapamil or diltiazem found to provide synergistic cardioprotective effect.
SMB Asdaget al Pharmacodynamic interaction of Calcium channel Blockers with garlic during Isoproterenol induced Myocardial Damage in Rat

	CKMB ACTIVIT	Δ.	LDH ACTIVITY		Heart tissue hom	ogenate	
Treatment	Serum (U/1)	HTH (U/g)	Serum (U/I)	HTH (U/g)	SOD (Units/mg protein)	CATALASE (Units/mg protein)	Histological scores
Normal control	11.90 ±1.46	210.40±2.78	324.96±16.85	16.20 ± 2.19	14.44±0.15	6.56 ± 0.04	0.5 ± 0.22
ISO control	$92.33 \pm 2.22^{***}$	$34.20{\pm}1.30$ ***	643.80 ± 3.14 ***	$3.40{\pm}0.72$ ***	4.10 ± 0.07 ***	2.04 ± 0.005 ***	$2.66\pm0.21^{***}$
GH-250	$58.03 \pm 8.69^{****}$	148.3±7.00 ***	398.60±11.63"	13.50±0.20"	16.96±0.49 *****	6.45±0.11"	0.5 ± 0.22 ***
GH-500	81.20 ± 1.27 ***	41.80 ± 1.60 ***	462.66 ± 15.07 ****	9.53±1.21***	16.23±0.42****	4.86±0.12 [*] ···	1.5 ± 0.34 ***
Nifedipine (NE)	$89.93\pm7.06^{***}$	27.66 ± 1.52 ***	599.90 ± 16.33 ***	4.73±1.61**	7.45±0.43***	1.48±0.11***	1.5 ± 0.24 **
GH-250 +NE	51.56± 4.33 ****aa	137.46 ± 4.80 * maa	465.00 ± 24.24 *** m aaa	12.00 ± 0.86 ************************************	14.81±0.50 *** ••• aaa	5.63±0.05 ^{™ aaa}	2.83±0.33 *** ••
GH-500 + NE	71.03± 3.59 *** a	$111.18\pm 2.33^{**a}$	419.40±15.26 ***•••***	9.33±1.47 ***** aa	12.15±0.10 *** *** aaa	4.35 ± 0.04 " aaa	1.0 ± 0.20 ^{aaa}
Verapamil (VL)	52.16 ± 3.14 ****	127.33 ± 8.57 ****	$506.63\pm 29.37^{***}$	$11.63\pm1.87^{**}$	$6.54{\pm}0.40$ ***	2.82 ± 0.26 ***	1.5±0.12 ^{**} ^{aa}
GH-250 + VL	28.86 ± 1.73 * maa	167.08 ± 2.11 *··· aa	208.60 ± 10.69 * maa	20.90 ± 2.07 " ^a	18.47±0.22 ***********************************	7.56 ± 0.09^{maaa}	$1.0 \pm 0.10^{*}$
GH-500 + VL	35.33 1.42 ***** ^{aa}	143.60 ± 1.73 ****a	356.40 ± 15.88 ^{m aa}	13.70±1.80"	16.87±0.15 *** ••• aaa	5.89±0.32 ^{™ aaa}	1.5 ± 0.22 ****
Diltiazem (DM)	30.30 ± 0.15 ****	95.90 ± 3.06 ***	486.68 ± 11.08 ***	9.90±1.21 ^{*••}	7.03±0.29 ***	2.85 ± 0.04 ***	$1.0 \pm 0.27^{**}$
GH-250 + DM	28.10±2.98 ***	$145.50\pm6.17^{**}$ and 32.50 ± 6.12	$261.81 \pm 18.55^{*maa}$	15.50 ± 0.34 " ^a	18.58 ± 0.27 ************************************	$8.28{\pm}0.08$ **** aaa	$1.0\pm 0.10^{****}$
GH-500 + DM	29.00 ± 3.59 ****	$122.02\pm 5.94^{**aa}$	323.70 ± 50.16 ^{aa}	10.90 ± 0.20 *"	$16.05\pm0.15^{*aa}$	5.92±0.04" aaa	1.5 ± 0.26 ***
All values are mean [±] compared to NE,	SEM, n=8; * P<0.05, ** P<0 VL and DM (comparison betv treatment p.o. + sever	0.01, *** P< 0.001 when comp ween NE/VL/DM Vs NE/VL/DM n days of NE/VL/DM p.o At th	ared to CONTROL; P<0.05, "P <br M+ GH). In GH groups-30 days c he end of treatment, all groups e	0.01, "'P< 0.001 when com of GH p.o.; in NE/VL/DM grc except Normal control, were	pared to Isoproterenol (ISO) c oup-7 days of NE/VL/DM p.o. subjected to 2 doses of ISO	control; ^a P<0.05, ^{aa} P<0.01, and in interactive groups- 150 mg/kg s.c	^{ªª} P< 0.001 when 30 days of GH

Table	2: Effect on electrocardio	graphic parameters and hean	nodynamic findings durir	ng myocardial damage ind	uced by isoproterenol (ISC	() in rats
	Heart rate	Body weight	HW/BW	QRS duration	QT segment	RR interval
Treatment	(beats/min)	(g)	(mg/g)	(ms)	(ms)	(ms)
Normal control	280 ±20	233±3.3	1.9 ± 0.05	15.21±1.11	15.11±1.43	14.34±1.23
ISO control	$340{\pm}20$	$193\pm1.6^{*}$	$2.1{\pm}0.05*$	19.51±2.13**	$13.10\pm1.12^*$	$12.21 \pm 1.31^{*}$
GH-250	260±14*	218±10.1"	2.4±0.01 ***	$18.21{\pm}1.10^{**}$	15.10±1.92	14.11±1.30
GH-500	250±23 *···	250±10.1 *****	2.2 ± 0.03 *	18.90 ± 1.97 **	16.12±0.23"	14.21±1.23
Nifedipine (NE)	$180{\pm}23$ *****	173±3.3 ***···	1.9±0.05	19.67±1.91 **	20.10±1.19 *••	12.11±1.34"
GH-250 +NE	$240{\pm}21$ *•••aa	251±7.2 ***••• aaa	$2.2\pm0.05^{*}$	15.43 ± 2.10^{maaa}	$18.11\pm0.11^{*a}$	15.23±1.41 ^{°aa}
GH-500 + NE	238 ± 28 *	235±5.0 ^{***} ^{***} ^{aaa}	$2.16{\pm}0.03$	16.98 ± 1.98 *** aa	$17.13\pm0.10^{**aa}$	14.43±1.54 ^{man}
Verapamil (VL)	250±15* [*] "	276±6.6*****	$2.14{\pm}0.03$ *	15.11±1.11"	15.16±1.11	15.13±1.16"
GH-250 + VL	$224{\pm}20^{**{-}1}$	256±12.0****	2.32±0.02*	14.76 ± 1.08 ****	14.31 ± 0.14 °a	14.23±1.18"
GH-500 + VL	220±25*"	245±5.0***••	$2.04{\pm}0.04^{*}$	$16.21 \pm 1.11^{*na}$	15.11±1.43	15.34±1.23"
Diltiazem (DM)	260±24 [*] "	280±2.8**••	$2.23{\pm}0.02$ *	16.62 ± 6.60 ****	15.13±0.10	14.17±1.06"
GH-250 + DM	224 ± 26 * ^{m aaa}	298±4.4*"	2.3 ± 0.04 *	$15.20\pm4.30^{**ma}$	14.16±1.11 ^{°a}	15.03 ± 0.80 "
GH-500 + DM	$225\pm 21^{*1}$	290±7.63 ***·"	2.29±0.06 [*] "	16.38±3.30"	15.11±1.14	14.84±1.23"
All values are mean: 0.001 when compare groups-30 ds	±SEM, n=8; 'P<0.05, "P<0.01 :d to NE, VL and DM (compar ₃ys of GH treatment <i>p.o.</i> + se [•]	1, "P< 0.001 when compared to C rison between NE/VL/DM Vs NE/ wen days of NE/VL/DM <i>p.o.</i> . At th	CONTROL; "P<0.05, "P<0.01 VL/DM+ GH). In GH groups- ie end of treatment, all group	, P< 0.001 when compared t 30 days of GH <i>p.o.</i> ; in NE/VL/I \s except Normal control, were	 Isoproterenol (ISO) control; DM group-7 days of NE/NL/DM subjected to 2 doses of ISO 	P<0.05, [™] P<0.01, [™] P< <i>M p.</i> 0. and in interactive 150 mg/kg s.c

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Fig.1: H&E (×100) stained microscopic section of normal

Fig.2a: H&E (×100) stained microscopic section of isoproterenol (ISO) control. There is loss of cellular architecture, necrosis and inflammation and with prominent fibrosis.



Fig. 2b: H&E (×100) microscopic section of heart tissue of animals pretreated with GH 250 mg/kg for 30 days orally before subjecting for isoproterenol (ISO). Normal cytoarchitecture of myocardium is seen.



Fig.2c: H&E (×100) microscopic section of heart tissue of animals pretreated with GH 500 mg/kg for 30 days orally before subjecting for isoproterenol (ISO). Normal cytoarchitecture of myocardium with mild increase in interstitial cavity is evident.



Fig.2d: H&E (100) microscopic section of heart tissue of animals pretreated with GH 250 mg/kg for 30 days orally followed by diltiazem for seven days orally before subjecting for isoproterenol (ISO). Complete protection to myocardium is observed with intact structural integrity.



Fig. 2e: H&E (×100) microscopic section of heart tissue of animals pretreated with

(I) GH 250 mg/kg for 30 days orally followed by nifedipine for seven days orally before subjecting for isoproterenol (ISO). Normal architecture is seen



(ii)Nifedipine administered for seven days and subsequently subjected to ISO damage. Loss of cellular architecture, necrosis and inflammation and with prominent fibrosis is evident.



CONCLUSION

In conclusion, combined prophylactic therapy of garlic with verapamil/diltiazem shows synergistic cardioprotective effect in animals subjected to myocardial stress. Moreover, nifedipine induced worsened myocardial damages in ISO subjected animals could be prevented in animals previously treated with garlic. However, further studies should be carried out to determine the influence of specific active constituent of GH when combined with CCBs in animals subjected to myocardial damage. We hope that this type of study will open new areas of research for interaction and counteraction between herb and conventional drugs when they are taken concurrently.

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Fig. 2f: H&E (×100) microscopic section of heart tissue of animals pretreated with GH 250 mg/kg for 30 days orally followed by verapamil forseven days orally before subjecting for isoproterenol (ISO). Normal cytoarchitecture of myocardium with mild increase in mild necrosis is evident



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Formulation and Evaluation of Orodispersible Tablets of Lansoprazole

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The orodispersible tablet is an alternative to oral liquid dosage form and is an ideal for the administration of drug in an unfavorable conditions where water is not available. Orodispersible tablets are disintegrated dissolved or suspended by saliva in the mouth and resulting in to easy swallowing of drug which can provide significant benefits to the paediatric and geriatric population as well as others who prefer the convenience of easily swallowable dosage forms. So, the patient compliance to the therapy is increased. Ulcer is an inflamed portion of the gastro intestinal tract which occurs due to the exposure of GIT to the gastric acid, this causes severe irritation to the GIT and this needs an immediate treatment. Ulcer can be treated either by the reduction in gastric acid secretion or neutralization of gastric acid, Lansoprazole is a proton pump inhibitor and inhibits the gastric acid secretion. So, in order to treat the ulcer immediately and effectively, in the present research work lansoprazole orodispersible tablets were prepared using sodium starch glycolate by direct compression method. The tablets prepared were found to be good without any chipping, capping and sticking. The hardness of the tablets is 3.3 ± 0.1 kg/cm² and they are weighing within a limit of \pm 7.5%. The water absorption ratio was 24.9 to 100.72% and the tablets were dispersed in between 15 to 24 seconds. The drug was uniformly dispersed in all the formulations in the range of 99.1% to 102.5%, among the formulation prepared, the tablet containing 5% of sodium starch glycolate releasing 100.995 \pm 0.230% of the drug within 26 min. The preparation of lansoprazole orodispersible tablets using sodium starch glycolate fulfilling the objectives of the present research work.

Keywords: Orodispersible tablets, lansoprazole, sodium starch glycolate, In-vitro drug release.

INTRODUCTION^{1, 2, 3}

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The concept of fast dissolving drug delivery system emerged from the desire to provide patient with conventional mean of taking their medication. Difficulty in swallowing (dysphasia) is a common problem of all age groups, especially elderly and pediatrics because of physiological changes associated with these groups of patients. Orodispersible tablets disintegrated, dissolved in or suspended by saliva in the mouth resulting in easy swallowing of the drug and provide significant benefits to the pediatric and geriatric population as well as other patients who prefer the convenience of easily swallowable dosage forms. Due to the rapid disintegration, dissolution and complete absorption the bioavailability of drug from this delivery system is significantly greater than conventional tablet. Orodispersible tablets combines the properties of solid dosage forms (stability) and the liquid dosage form (absorption). So, the fast dissolving tablet is an important and attractive dosage form and ideal for unfavorable conditions of administration where water is not available.

MATERIALS AND METHODS

Lansoprazole was obtained as a gift sample (Batch no.LSFP10014) from Lee Pharma Limited Hyderabad and

RGUHS Journal of Pharmaceutical Sciences Received: 28/01/2011, Modified: 21/02/2011, Accepted: 27/02/2011 sodium starch glycolate (Lot. No.E0630) is a gift sample from Signet Chemical Corporation Pvt. Ltd., Mumbai. All other exipients are pharmacopoeial grade.

Preparation of orodispersible tablets:^{4,5}

Lansoprazole orodispersible tablets were prepared by direct compression method. All the ingredients were powdered separately in a clean and dry porcelain mortar and then they were passed through # 60 mesh sieve. The drug and the additives were mixed thoroughly in an inflated polyethylene pouch in a geometric ratio of their weight and then the powder mixture was compressed in to tablets of 240 mg weight using 8 mm flat round punches. The detailed composition of the formulation is shown in table 1.

Evaluation of Lansoprazole orodispersible tablets

The lansoprazole orodispersible tablets were evaluated for the following parameters.

1. Hardness

Three tablets were selected from each batch and they were subjected to hardness test using Monsanto hardness tester and the results were shown in table-2.

2. Thickness and Diameter⁷: Three tablets were selected from each batch and they were subjected to the thickness and diameter test using vernier calipers, the result were shown in table-2.

3. weight variation (Uniformity of weight)⁷:

From each batch twenty tablets were selected randomly, weighed them individually and the average weight was determined. The percentage deviation of individual tablet was calculated from the average weight.

4. Wetting time and water absorption ratio^{8,9}:

The wetting time of the tablets was measured by the simple procedure. Five circular tissue papers of 10 cm diameter were placed in a petridish, a tablet was carefully placed on the surface of the tissue paper and the time required for water to wet the tablet completely (to reach upper surface of the tablet) was noted.

The water absorption ratio was determined by keeping preweighed tablet in the petridish then the tablet was allowed to absorb water then it was taken out and reweighed.

Water absorption ratio 'R' was determined using following equation

$$R = 100 x \begin{pmatrix} W_b - W_a \\ - - - - - \\ W_a \end{pmatrix}$$

Where, W_a is weight of tablet before water absorption and W_b is weight of tablet after water absorption. The water absorption ratio was shown in Photograph 3.

5. Drug content uniformity¹⁰:

Five tablets were weighed and powdered; a quantity of powder equivalent to 10 mg of drug was dissolved in 100 ml of methanol (100 µg/ml). 0.9ml solution was pipetted from this stock solution and volume was made to 10 ml using methanol to give a concentration ≈ 9 g/ml, the absorbance of the resulting solution was measured at 285.7 nm against methanol as a blank. The amount of drug present in the solution was calculated with the help of standard calibration curve. The procedure was repeated for three times and the mean percentage drug content was calculated. The results were shown in Table-2.

6. *In-vitro* **dispersion time**¹¹: *In-vitro* dispersion is the main evaluation parameter for orodispersible tablets and it was determined by adding a tablet to 10 ml of pH 6.8 phosphate buffer solution at $37\pm0.5^{\circ}$ C. Time required for complete dispersion of a tablet was noted and the results were shown in table 2 and Photograph 1 and 2.

7. *In-vitro* Release study¹²:

In vitro drug release from lansoprazole orodispersible tablet was studied employing USP XXIII type-II dissolution apparatus (Electro lab TDT-06N) using paddle stirrer. 900 ml of pH 6.8 phosphate buffers with 5 ml SLS was used as dissolution medium, the temperature of dissolution medium was maintained at $37\pm0.5^{\circ}$ C throughout the experiment and the paddle was allowed to rotate at 50 rpm. 5 ml samples of dissolution medium were withdrawn by means of syringe fitted with pre-filter at known intervals of time and analyzed for drug release for each time interval by measuring the absorbance at 285.5 nm after suitable dilution. The volume withdrawn at each time interval was replaced with fresh quantity of dissolution medium. Cumulative percent drug release was calculated and the release data was plotted for various graphs.

RESULTS AND DISCUSSION

Fast dissolving tablets are of very much useful in the treatment of acute conditions and the literature suggests the use of superdisintegrants in the preparation of orodispersible tablets for the faster dispersion and the dissolution of the tablet.

In the present research work lansoprazole orodispersible tablets were prepared using 2%, 3%, 4% and 5% of sodium starch glycolate by direct compression method. The tablets were evaluated for various parameters like hardness, thickness, weight variation, drug content uniformity, water absorption ratio, wetting time, *In-vitro* dispersion time and *In -vitro* drug release and the results were shown in the table 2.

Hardness of the tablet prepared was in the range of 3.3 ± 0.1

Table 1: Formulation de	etails of	orodispei	sible tab	let of lans	soprazole
Ingredient (mg/tab)	F1	F2	F3	F4	F5
					(controlled)
Lansoprazole	30	30	30	30	30
Sodium starch glycolate	4.8	7.2	9.6	12	-
Microcrystalline cellulose	72	72	72	72	72
Mg.Stearate	2.4	2.4	2.4	2.4	2.4
Talcum	2.4	2.4	2.4	2.4	2.4
Mannitol	2.4	2.4	2.4	2.4	2.4
Lactose	126	123.6	121.2	118.8	130.8
TOTAL	240	240	240	240	240



Photograph-3: water absorption ratio of lansoprazole orodispersible tablet prepared using sodium starch glycolate 5%



to 3.8 ± 0.1 kg/cm² indicating good mechanical strength of the tablet. The tablets of the each batch were subjected to weight variation test and the result suggests that all the tablets are weighing within the permissible percentage deviation of $\pm7.5\%$.



The tablets were also evaluated for water absorption ratio, wetting time and *In-vitro* dispersion, the result reviews that the water absorption ratio for the tablets prepared using 2%, 3%, 4% and 5% of sodium starch glycolate was 24.9 \pm 4.2%, 39.5 \pm 2.1%, 59.5 \pm 1.6% and 107.6 \pm 3.2% respectively. The wetting time for the tablets prepared using 2%, 3%, 4% and 5% of sodium starch glycolate was 28.3 \pm 0.57 sec, 26.7 \pm 0.57 sec, 24.6 \pm 0.58 sec and 20.7 \pm 0.57 sec respectively. The *In-vitro* dispersion time for the same tablets was 24 \pm 1 sec, 22.3 \pm 0.6 sec, 18.7 \pm 0.6 sec and 15.7 \pm 0.6 sec respectively. The tablet containing 5% of sodium starch glycolate showed rapid dispersion (15.7 \pm 0.6 sec), this improved *In-vitro* dispersion may be due to the higher percentage of water absorption ratio and lower value of the wetting time/immediate wetting of the tablet.

The lansoprazole tablets prepared were also evaluated for drug content uniformity test, the drug was uniformly dispersed in the range of 99.1 ± 0.4 to $102.5\pm0.8\%$ through out all the formulations (USP limit is $\pm 10\%$) and the low value of standard deviation indicates the accuracy in the procedure followed.

The lansoprazole tablets prepared were also evaluated for *In-vitro* drug release studies, the orodispersible tablets

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Formulation	Hardness	Diameter	Thickness	Weight	Drug content	In-vitro	Wetting	Water	Diss	olution Pr	ofile
	(Kg/cm^2)	(uu)	(mm)	Variation (%)	uniformity (%)	dispersion time(sec)	time(sec)	absorption Ratio(%)	$\overline{\mathbf{t}}_{50\%}$ (min)	$\mathbf{t}_{75\%}$ (min)	t _{90%} (min)
Fl	3.3 ± 0.1	8	3.9 ± 0.1	°/0°.7	99.1 ± 0.42	24±11	28.3 ± 0.57	24.9±4.23	17.2	27.36	35.36
F2	3.3 ± 0.1	8	3.9 ± 0.1	°/0°.7.2	99.9 ± 0.51	22.3 ± 0.60	26.7 ± 0.57	39.5 ± 2.12	16.4	26	30.48
F3	3.3 ± 0.1	8	3.9 ± 0.1	°/0°.7.2	102.5 ± 0.81	18.7 ± 0.62	24.6 ± 0.58	59.5 ± 1.63	15.6	24	20.48
$\mathrm{F4}$	3.3 ± 0.1	8	3.8 ± 0.1	°%2.7.5	100.5 ± 0.94 1	15.7 ± 0.61	20.7 ± 0.57	107.6 ± 3.21	8	14.12	19.24
F5 (controlled)	3.8 ± 0.1	8	3.9 ± 0.1	°%2°.2	99.4±1.12	720 ± 1.22	1446 ± 0.58	22.7 ± 2.52	42	48.36	59.40

containing 2%, 3%, 4% and 5% of sodium starch glycolate releasing 92.382 \pm 0.237%, 98.995 \pm 0.152%, 100.912 \pm 0.161% and 100.995 \pm 0.230% drug at the end of 36th min, 36th min, 36th min and 26th min respectively. The release data were plotted for release plot (fig-1) and first order plots (fig-2). The formulation containing 5% of sodium starch glycolate (F4) releasing 100.995 \pm 0.230% of the drug within 26 min. This improved/increased drug release may be due to the higher percentage of water absorption, minimum wetting time and lesser *In -vitro* disintegration time.

CONCLUSION

In the present research work, lansoprazole orodispersible tablets were prepared using 2%, 3%, 4% and 5% of sodium starch glycolate by direct compression method. The tablets were subjected to hardness, weight variation, drug content uniformity, water absorption ratio, wetting time, *In-vitro* dispersion time and in vitro drug release studies. The results of the above parameters are within the permissible limits and the tablet containing 5% of sodium starch glycolate showed a very good and encouraging results. So the preparation of lansoprazole orodispersible tablets using sodium starch glycolate fulfilling the objectives of the present research work.

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Visible Spectro Photometric Determination of Darifenacin Hydrobromide in Bulk and Pharmaceutical dosage forms

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- A simple and sensitive visible spectro photometric method has been developed for the quantitative estimation of Darifenacin hydrobromide in bulk drugs and pharmaceutical dosage forms. It involves diazotization of Darifenacin hydrobromide by sodium nitrite in acidic medium followed by complexation with starch $\$ KI reagent to yield a bluishpurple colored complex, which shows maximum absorption at 620 nm against distilled water as blank. The results of the analysis were found to be 99.67% and 98.95% for assay with a % RSD ranging from 0.301-0.422%. The mean % recovery for formulation first is 99.36 \pm 0.491% and for that of second formulation is 99.78 \pm 0.235%.
- Keywords: Darifenacin hydrobromide, Visible spectro photometry, Diazotization, Recovery, Assay.

INTRODUCTION

Darifenacin hydrobromide (s)-2-{1-[2-(2, 3dihydrobenzofuran-5-yl)ethyl 3-pyrolidine}-2diphenyl acetamide hydrobromide (**Figure 1**), is a novel muscarinic receptor antagonist developed for the treatment of overactive bladder (OAB). Overactive bladder is characterized by symptoms of increased frequency of micturition, urgency and urge incontinence [1]. The symptomatic treatment of the urge incontinence and/or increased urinary frequency and urgency as may occur in patients with overactive bladder syndrome using Darifenacin hydrobromide is also studied [2].

Darifenacine hydrobromide is a potent inhibitor for the contractile responses of induced over active bladder. In this study twenty four male and female rabbits were utilized. It showed equal potency in both sexes and had no side effects such as effect on blood pressure etc [3]. Darifenacine hydrobromide displays up to 59-fold selectivity for M3 receptors relative to other muscarinic receptor subtypes in vitro [4].

No reports were found in the literature for the quantitative estimation of Darifenacin hydrobromide by HPLC, HPTLC and spectrophotometry. In the present work, a simple and sensitive visible spectrophotometric method has been developed for the quantitative estimation of Darifenacin hydrobromide in bulk drug and Pharmaceutical dosage forms.

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MATERIALS AND METHODS

The instrument used in the present study was JASCO double beam UV-Visible spectrophotometer (Model V-550) with slit width fixed at 2 nm connected to a computer with spectra manager software. All weighing were done on electronic balance (Model Shimdzu AY 120).

Starch/KI reagent (1 g of starch and 0.5 g of KI added to 20 mL of distilled water, heated till clear, transferred to 50 mL volumetric flask and volume made up to 50 mL with distilled water). The commercially available tablets of Darilong 7.5 and Darilong 15 tablets were procured from a local pharmacy. Darifenacin hydrobromide (analyzed sample) as provided by Ranbaxy limited was used as such without further purification.

Preparation of standard stock solution:

A solution of Darifenacin hydrobromide was prepared by

dissolving 10 mg (accurately weighed) of the standard in 100 mL of 0.1 N Hydrochloric acid. From the stock solution aliquots (0.5, 1, 1.5, 2 and 2.5 mL) were transferred into a series of 10 mL volumetric flasks. To each volumetric flask, added 0.1 % NaNO₂ (2 mL) and 2 mL of starch / KI reagent and volume was made up with distilled water and kept aside for 15 min. Diazotization of Darifenacin hydrobromide by sodium nitrite in acidic medium followed by complexation with starch \KI reagent to yield a bluish - purple colored complex. The absorbance of bluish-purple chromogen was measured at 620 nm against distilled water as blank and calibration curve was plotted.

Preparation of sample stock solution:

For tablets, average weight of twenty tablets was determined and these were then finely powdered. The powered amount equivalent to 10 mg of tablets was dissolved in 100 ml 0.1 N Hydrochloric acid separately, shaken for 10 minutes and filtered. One mL of this solution was taken in 10 mL volumetric flask, to this added 2 mL of 0.1 % NaNO₂ and 2 mL of starch / KI reagent and volume made up to mark with distilled water and kept aside for 15 min. The absorbance of this solution was measured at 620 nm against distilled water as blank. The concentration of drug is determined by calibration curve method.

Assay of the Darifenacin in Prepared formulation:

To determine the content of Darifenacin hydrobromide in tablet: Ten tablets of of Darifenacin hydrobromide were weighed, their average weight was determined, and finally they were crushed to a fine powder. The tablets powder equivalent to 5mg of Darifenacin hydrobromide were weighed and transferred into a 10 mL volumetric flask and dissolved in 5mL of methanol and volume was made up to the mark with methanol. The content was ultrasonicated for 30 min for complete dissolution.

The solution is filtered through Whattman filter paper No 41 and 2mL of solution was added 2 mL of $0.1 \% NaNO_2$ and 2 mL of starch /KI reagent and volume made up to mark with distilled water and kept aside for 15 minutes. The absorbance of this solution was measured at 620 nm against distilled water as blank. The concentration of drug is determined by calibration curve method.

Recovery:

To carry out the recovery studies the analyzed samples were spiked with extra 5, 10 and 15 % of the standard Darifenacin hydrobromide and the mixture was subjected to reanalysis

by the proposed method. The experiment was conducted in triplicates. This was done to check for the recovery of the drug at different levels in all the formulations.

RESULTS AND DISCUSSION

The operating conditions used in the procedure were Optimised by adopting the variations of one variable at a time. The absorption maximum of the chromogen was found to be 620 nm, as shown in **Figure 2.** Recovery experiments were carried to test the accuracy and reproducibility of the proposed method, by adding known amount of the drug to the preanalysed formulation and reanalyzing the mixture. Stability study of the chromogen was carried out by measuring the absorbance of chromogen in time scan mode and was found to be stable for 2 hours. The optical characteristics such as absorption maxima, Beer's Law limits, correlation coefficient (r), slope (m) and yintercept (c) were calculated from 5 replicate reading are incorporated in **Table 1**.

Assay of commercial formulations:

The absorbance of this solution was measured at 620 nm against distilled water as blank. The concentration of drug is determined by calibration curve. There was no interference from the excepients commonly present in the tablets. The Darifenacin content was found to be **99.67%** and **98.95%** with a % RSD ranging from **0.301-0.422%**.

It may therefore be inferred that degradation of Darifenacin hydrobromide had not occurred in the formulations that were analyzed by this method. The low % RSD values indicated the stability of this method for routine analysis of Darifenacin in pharmaceutical dosage forms (**Table 2**).

Recovery:

The proposed method when used for the extraction and subsequent estimation of Darifenacin hydrobromide for Pharmaceutical dosage forms after spiking with 5, 10 and 15 μ g of additional drug afforded recovery of 99.36 \pm 0.491% in the first formulation and 99.78 \pm 0.235% in second formulation as listed in **Table 3** and **4** respectively.

CONCLUSION

The Visible spectrophotometry method proposed is particularly appropriate for the routine analysis of darifenacin hydrobromide in tablet dosage form. This method has the advantages of simplicity, precision, accuracy, sensitivity and quantification of darifenacin hydrobromide in bulk and pharmaceutical dosage forms. NK Sathish et al Visible Spectro Photometric Determination of Darifenacin Hydrobromide in bulk and Pharmaceutical dosage forms



	Table 2: A	ssay of commercia	I formulations.		
Formulation	Amount labeled (mg)	Amount found* (mg)/tab	% Assay	S.D.*	% RSD*
DARILONG 7.5 Tablets	7.5	7.475	99.67	0.421	0.422
DARILONG 15 Tablets	15	14.842	98.95	0.298	0.301

*Average of six determinations. S.D. is standard deviation, RSD is relative standard deviation.

	Ta	able 3: Recovery study t	for tablet formulatio	on (l).	
Drug name	Amount	Amount of	Total	%	Mean %
	label	standard	amount	Recovery	Recovery
	claimed	added	recovered	*	(± S.D.)
	(µg∕ml)	(µg∕ml)	(μg/ml)		
Darifanaain	10	5	14.768	98.45	
HBr (Tablet)	10	5	14.918	99.45	99.36 ± 0.491
(10	5	14.88	99.2	
DARILONG	10	10	19.932	99.66	
7.5	10	10	19.826	99.13	
	10	10	19.998	99.99	
	10	15	24.745	98.98	
	10	15	24.863	99.45	
	10	15	24.993	99 97	

	Table	4: Recovery studies	for tablet formulatior	n (II).	
Drug name	Amount label claimed	Amount of standard added	Total amount recovered	% Recovery *	Mean % Recovery (± S.D.)
	(µg/ III)	(µg/ III)	(µg/ IIII)		
	10	5	14.892	99.28	
Darifenacin	10	5	14.933	99.55	
HBr (Tablet)	10	5	14.964	99.76	99.78 ± 0.235
DARILONG	10	10	19.952	99.76	00110 - 01200
15	10	10	19.992	99.96	
	10	10	19.996	99.98	
	10	15	24.985	99.94	
	10	15	24.948	99.79	
	10	15	24.995	99.98	
	* Avg.	of three determinations,	S.D. is standard deviati	on.	

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Antihyperglycemic and Antihyperlipidemic effect of *Rubia cordifolia* leaf extract on Alloxan-induced Diabetes

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The study was designed to investigate the effect of alcohol extract of leaves of *Rubia cordifolia* Linn. in normal and alloxan-induced diabetic rats. *Rubia cordifolia* alcohol extract was evaluated for its acute toxicity in female mice. Two doses of *Rubia cordifolia* alcohol extract were selected and were evaluated for antidiabetic activity in alloxan-induced diabetic male rats and for hypoglycemic activity in normal fasted rats. In normal treated rats both the doses of *Rubia cordifolia* alcohol extract, 200 and 400 mg/kg decrease (d) the blood glucose level by 32.15 and 39.02%, respectively compared to glibenclamide (600 g/kg) by 45.72% after 15 days of treatment. In addition, the extract also showed a favorable effect on glucose disposition in glucose-fed hyperglycemic rats after 90 min of glucose administration. In alloxan-induced diabetic rats both the doses of *Rubia cordifolia* alcohol extract decreases the blood glucose level by 48.46 and 51.56%, respectively compared to the glibenclamide by 57.28% after 15 days treatment. Serum cholesterol and triglyceride level were decreased whereas serum high-density lipoprotein and protein levels were increased in diabetic rats. The histopathological study showed the regeneration of β-cell in both extract and glibenclamide treated groups. The alcohol extract of *Rubia cordifolia* leaves have a promising antidiabetic activity against alloxan-induced diabetic rats.

Keywords: Alloxan, diabetes mellitus, glibenclamide, Rubia cordifolia

INTRODUCTION

Diabetes mellitus is a common metabolic disorder associated with increased morbidity, mortality rate and can be defined as a group of metabolic diseases characterized by chronic hyperglycemia due to defect in insulin secretion, insulin action or both, resulting in impaired carbohydrate, lipid and protein metabolism¹. Diabetes is a major health problem worldwide; approximately 5% of the world's population suffers from diabetes. Since diabetes mellitus is a multifactorial disease, the treatment is aimed not only decreasing the blood sugar level to normal limit, but also at correcting the metabolic defects associated with it. According to the WHO projection, diabetic population is likely to increase over 300 million by the year 2025. Currently available therapies for diabetes include insulin and oral antidiabetic agents such as sulfonylureas, biguanides, and α -glycosidase inhibitors, which are used either as a monotherapy or in combination to achieve better glycemic control. Many of these oral antidiabetic agents have a number of serious adverse effects that make the management of diabetes a great challenge².

So there is an increasing demand by patient to use the natural product with antidiabetic activity. This is because

RGUHS Journal of Pharmaceutical Sciences Received: 29/01/2011, Modified: 21/02/2011, Accepted : 28/02/2011 insulin can not be used orally and continuous insulin injections have many side effects and toxicity. Besides it, certain oral hypoglycemic agents are also not effective in lowering the blood sugar in chronic diabetic patients³. The antihyperglycemic and antioxidant property of the roots of *Rubia cordifolia* Linn. has been previously reported^{4,5}. Traditionally aerial part of the plant shows hypoglycemic activity and reports available for use in diabetic microangiography^{6,7}. Therefore, the present study has been undertaken to evaluate the effect of *Rubia cordifolia* Linn. leaf extract on blood glucose level in normal and alloxaninduced diabetic rats to give a scientific background to the above traditional claim.

MATERIALS AND METHODS

Plant material and preparation of alcoholic extract:

Fresh leaves of *Rubia cordifolia* were collected from Sirsi hills in Karnataka, in the month of June. The plant was authenticated at the H. S. Kotambri Science Institute, Hubli. The coarsely powdered form of shade-dried leaves were subjected to exhaustive extraction in a Soxhlet apparatus using 95% alcohol. The extract was concentrated in a rotavap and residue was dried in desiccator over sodium sulphite. The semisolid mass obtained was stored in an air tight container in refrigerator for further use. The extract was suspended in 5% Tween-80 and used by oral administration for the acute toxicity and antidiabetic activity evaluations.

Animals and housing parameters:

Female Swiss mice (20-25 g) for acute toxicity and male Wistar rats (150-200 g) for antidiabetic were used throughout the experiments. The animals were procured from animal house of K. L. E. S. College of Pharmacy, Hubli. Standard environmental conditions such as temperature $(26\pm2^{\circ})$, relative humidity (45-55%) and 12 h dark/light cycle were maintained in the quarantine. All the animals were fed with rodent pellet diet (Gold Mohr, Lipton India Ltd. Mumbai) and water was allowed *ad libitum* under strict hygienic conditions. Ethical clearance for performing the experiments on animals was obtained from Institutional Animal Ethics Committee (IAEC). After randomization into various groups and before initiation of experiment, the animals were acclimatized for a period of 7 days.

Toxicity evaluation in mice:

Randomized female Swiss mice were marked for individual identification. Animals were fasted overnight prior to dosing. Test substances were administered in a single dose orally. No mortality and no signs of toxicity were found even after administration of a limit dose of 2000 mg/kg of extract, as per OECD guideline No. 425 the substance might be considered to have an LD_{50} value above 5000 mg/kg body weight⁸. Two doses, 200 and 400 mg/kg were selected for the present study.

Glucose tolerance test:

Fresh normal animals were used for oral glucose tolerance test (OGTT). The overnight fasted rats were divided into four groups of normal control, *Rubia cordifolia* alcohol extract (RCAE, 200 and 400 mg/kg) and glibenclamide (GLB, 600 g/kg) treated groups containing six rats in each group. Thirty minutes after giving the above treatments, the rats of all groups were orally treated with 2 g/kg of glucose⁹. Blood samples were collected prior to glucose administration and at 30 and 90 min after glucose loading. Blood sample withdrawals were done from retro-orbital plexus under light ether anesthesia. Plasma was separated and used for the estimation of blood glucose.

Hypoglycemic study in normal fasted rats:

The animals were fasted for 18 h but were allowed to water before and throughout the duration of experiment. At the end of fasting period, taken as zero time (0 h), blood was withdrawn (0.1 ml) from orbital sinus under light ether anesthesia and blood glucose level was estimated. The normal rats were then divided into four groups of six rats each. Group-I served as normal control which received only vehicle (2 ml/kg) through oral route. Group-II and III received the RCAE at a dose of 200 mg/kg and 400 mg/kg, respectively and Group IV received the reference drug GLB (600 g/kg, orally). Blood glucose levels were examined on 0, 1, 4, 7, 10, and 15th day of administration of test samples.

Alloxan-induced diabetic rats:

The selected animals were weighed and marked for individual identification. Diabetes was induced in rats that had been fasted for 12 h by intraperitoneal injection of 150 mg/kg of alloxan¹⁰, freshly dissolved in sterile normal saline before use to give a concentration of 30 g/l. Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20 % glucose solution intraperitoneally after 6 h. The rats were then kept for the next 24 h on 5 % glucose solution bottles in their cages to prevent hypoglycemia¹¹. After 72 h, blood glucose was measured colorimetrically in Star 21^{Plus} Biochemistry Auto-analyzer with reagents from Erba Diagnostics Kit. One millilitre of blood was taken from the orbital sinus of each rat with the help of a capillary tube for the estimation of blood sugar. Rats showing fasting blood glucose level around 300 mg/dl were selected for the study.

Determination of the efficacy of the plant extract in diabetic rats:

The alloxan-induced diabetic rats were divided into 4 groups of 6 each. Group I was given 1 ml of 5% Tween 80 p.o. daily and served as control. Group II and III were given RCAE (200 and 400mg/kg) respectively. Group IV received the GLB (600 g/kg). The treatment was continued for 15 days. Blood samples were collected in morning 1 h after drug administration on day 1, 4, 7, 10 and 15 day. On 15th day animals were sacrificed after blood collection under light ether anesthesia and pancreas were removed for histopathological study.

Estimation of biochemical parameters:

Blood glucose¹², serum total cholesterol¹³, serum HDL¹⁴, serum triglyceride¹⁵ and serum protein¹⁶ were measured colorimetrically in Star 21^{Plus} Biochemistry Auto-analyzer with reagents from Erba Diagnostics Kit.

Statistical Analysis:

Statistical evaluation was done using one-way analysis of variance (ANOVA). Post-hoc comparisons were done by using Dunnett test. p values < 0.05 were considered significant.

RESULTS

Effect of alcoholic extract on normal rats:

Oral administration of RCAE at doses equivalent to 200 and 400 mg/kg produced significant (p<0.05) hypoglycemic effect in normal fasted animals on 10th day. The RCAE showed optimum activity at 200 mg/kg and at a higher dose 400 mg/kg, it did not show a matching increase in activity. Both the doses of RCAE 200 and 400 mg/kg showed the maximum reduction of the blood glucose level on 10th day. On 15th day both the doses of RCAE reduced the blood glucose level but the effect was not much significant on 15th day as compared to the 10^{th} day. It is worthy to mention that animals treated with GLB (600 g/kg) showed a significant reduction of blood glucose level on 15th day. Oral administration of RCAE at a dose of 200 mg/kg produced significant hypoglycemic effect. However, the hypoglycemic effect was increased but was not proportionate with the administration of 400 mg/kg as outlined in Table 1.

Effect of alcoholic extract on OGTT:

Effects of RCAE on blood glucose level of normal fasted rats after a glucose load (2.0 g/kg) are outlined in Table 2. The blood glucose level increases rapidly 30 min after glucose

administration, and thereafter decreased gradually. When two different doses of the RCAE (200 and 400 mg/kg) were given orally before the glucose administration, it was found that though the dose of 400 mg/kg produced effect but was not significantly increased as compared to the dose 200 mg/kg which has shown optimal effect.

Effect of alcohol extract on diabetic rats:

The RCAE exhibited antidiabetic property in alloxaninduced diabetic rats as evident from serum glucose and significant improvement in lipid profile. The effect of the RCAE on serum glucose level in alloxan-induced diabetic rats is shown in Table 3. The effect of the extract on lipid profile and body weight in alloxan-induced diabetic rats is given in Table 4 and 5. The initial blood glucose levels of the diabetic rats selected for the study were in the range of 320 to 330 mg/100 ml. In the diabetic control rats, the blood glucose level increased to 355.42 ± 1.1030 mg/100 ml on the tenth day. On the 15th day blood glucose level decreased to a mean value of 342.50±1.3760. Both the doses of RCAE (200 and 400 mg/kg) steadily decreased the blood glucose level from an initial value of 323.45±0.6702 and 324.53±0.5270 to a mean value of 166.73±1.0460 and 157.21 ± 0.5799 respectively on 15^{th} day. Thus the drug

	Table 1: Effect of RC/	AE on blood glucose	e level in normal rate	6
Treatment	Non-diabetic Control (mg/dl)	RCAE 200 (mg/dl)	RCAE 400 (mg/dl)	GLB (mg/dl)
Before	85.18±0.60	84.27±0.10	84.75±1.30	85.43±0.79
1 st Day	82.45 ± 0.33^{ns}	81.12 ± 0.30^{ns}	$80.25 \pm 0.88^{\circ}$	76.17 ± 0.72^{a}
4 th Day	83.47 ± 0.27^{ns}	77.02 ± 0.48^{a}	$75.35 \pm 0.44^{\circ}$	$67.23 \pm 0.39^{\circ}$
7 th Day	83.67 ± 0.26^{ns}	$69.28 \pm 0.75^{\circ}$	$66.12 \pm 0.47^{\circ}$	$58.6\pm0.58^{\rm a}$
10 th Day	84.40 ± 0.49^{ns}	60.35 ± 0.62^{a}	$56.43 \pm 0.36^{\circ}$	$48.32 \pm 0.24^{\circ}$
15 th Day	85.45±1.21 ^{ns}	57.18 ± 0.47^{a}	51.68 ± 0.32^{a}	46.37 ± 0.33^{a}

Values expressed as mean±SEM, n=6 animals in each group. ns= not significant. The results were analyzed using One way ANOVA followed by Dunnett's multiple comparison tests. ^ap<0.05 was used to indicate statistical significance when compared to control.

Table 2: Effect of Rul	oia cordifolia extra	ts on blood glucos	e level (mg/dl) in nor	mal rats (ogtt)
Treatment	Control (mg/dl)	RCAE 200 (mg/dl)	RCAE 400 (mg/dl)	GLB (mg/dl)
Mean values prior to treatment	82.28±0.74	80.57±0.46	81.42±0.40	80.33±0.59
30 min after glucose loading	191.47±1.01	$181.38 \pm 0.39^{\circ}$	$177.57 \pm 0.18^{\circ}$	171.20 ± 0.24^{a}
90 min after glucose loading	110.23±0.68	$106.66 \pm 0.43^{\circ}$	$103.18 \pm 0.18^{\circ}$	$98.43 \pm 0.22^{\circ}$
Values are expressed tes	above as mean±SEM t. °p<0.05 compared t	1, n=6 in each group. C to normal control at the	One way ANOVA followe	ed by Dunnett

treatment restored the serum glucose level nearer to the normal values. The effect of RCAE is compared with the standard drug GLB (600 $\,$ g/kg).

As shown in Table 4, in diabetic control rats serum cholesterol and triglyceride level were increased and HDL and protein level were decreased. These diabetic complications were significantly attenuated with the administration of RCAE 200 and 400 mg/kg. The effect of the standard drug GLB on serum cholesterol, HDL, triglyceride and protein in diabetic rats were comparable to the RCAE.

The effect of the extract on body weight is given in Table 5. The body weight was slightly increased in normal rats compared to the initial body weight, whereas in the diabetic control rats there was a significant decrease in the body weight. GLB as well as RCAE (200 and 400 mg/kg) significantly prevented this reduction in body weight. Histopathological study of pancreas showed normal acini and normal cellular population in Islets of Langerhans and absence of both damage to Islets and hyperplasia in normal control rats (fig. 1a). In diabetic control rats Islet damage and reduced Islets size was seen (fig. 1b). In both GLB 600 g/kg (fig. 1c) and RCAE 400 mg/kg (fig. 1d) treated rats restoration of normal cellular population size of Islets of Langerhans was observed and also there was absence of Islet damage and presence of hyperplasia.

DISCUSSION

The present study is aimed at finding out if chronic administration of different doses of RCAE (200 and 400 mg/kg) would have any influence in normal and diabetic animal in comparison to standard GLB. Most studies were either of short duration (from h to a maximum of 5 days) or

	Table 3: Effect of R	CAE on blood gluco	ose level in alloxan-	induced diabetic ra	ıts
Treatment	Non-diabetic Control (mg/dl)	Diabetic Control (mg/dl)	RCAE 200 (mg/dl)	RCAE 400 (mg/dl)	GLB (mg/dl)
Before	85.18±0.59	325.62 ± 0.71^{a}	323.45 ± 0.67	324.53 ± 0.52	$323.78 {\pm} 0.46$
1^{st} Day	82.45 ± 0.32	332.72 ± 0.97^{a}	$304.72 \pm 1.4^{\text{b}}$	$302.51 \pm 1.16^{\text{b}}$	$289.32 \pm 1.21^{\circ}$
4 th Day	83.47±0.26	341.26 ± 0.69^{a}	$279.45 \pm 1.15^{\text{b}}$	$277.62 \pm 1.17^{\text{b}}$	$262.92 \pm 0.95^{\circ}$
7^{th} Day	83.67±0.26	348.15 ± 0.79^{a}	$248.31 \pm 0.93^{\text{b}}$	$245.12 \pm 1.16^{\text{b}}$	$238.63 \pm 1.24^{\text{b}}$
10 th Day	84.40 ± 0.49	355.42 ± 1.10^{a}	$209.65 \pm 0.96^{\circ}$	$206.5 \pm 0.93^{\circ}$	$188.55 \pm 1.11^{\text{b}}$
15 th Day	85.45±1.21	342.50±1.37 ^a	166.73±1.04 ^b	157.21±0.57 ^b	138.32±1.02 ^b

Values expressed as mean±SEM, n=6 animals in each group. The results were analyzed using One way ANOVA followed by Dunnett's multiple comparison tests. ^ap<0.05 compared to non-diabetic control; ^bp<0.05 compared to diabetic control on respective day.

Table 4: Effect of RCAE on serum lipid level in non-diabetic control and alloxan-induced diabetic rats						
Groups	Total Cholesterol (mg/dl)	HDL (mg/dl)	Triglyceride (mg/dl)	Protein (mg/dl)		
Non-diabetic control	127.42±1.02	36.42 ± 0.52	125.28 ± 0.78	7.15±0.12		
Diabetic control	269.70 ± 1.45^{a}	$26.62 \pm 0.51^{\circ}$	215.76 ± 0.51^{a}	4.04 ± 0.22^{a}		
RCAE 200 mg/kg	175.62±0.79 ^b	30.47 ± 0.57 ^b	153.47±0.94 ^b	5.14±0.03 ^b		
RCAE 400 mg/kg	165.12±0.72 ^b	32.68 ± 0.57 ^b	146.26±0.69 ^b	5.87 ± 0.04 ^b		
GLB 600 µg/kg	147.28±0.42 ^b	34.72±0.58 ^b	136.53±0.63 ^b	6.51±0.05 ^b		
Values expressed as mean±SEM, n=6 animals in each group. The results were analyzed using One way ANOVA						

followed by Dunnett's multiple comparison tests. *p<0.05 compared to non-diabetic control; *p<0.05 compared to diabetic control; *p<0.05 compared to diabetic control on respective day.

Table 5: Effect of RCAE on body weight in non-diabetic control and alloxan-induced diabetic rats								
Treatment	Non- diabetic	Diabetic	RCAE 200	RCAE	GLB			
	Control	Control	mg/kg	400/mg/kg				
Mean values								
before treatment	173.3±0.7	161.5 ± 0.42	160.5 ± 0.63	162.0±0.38	163.7±0.51			
7 th Day	184.2±0.3	150.8 ± 0.6^{a}	$167.8 \pm 0.47^{\circ}$	$170.5 \pm 0.42^{\text{b}}$	$177.2 \pm 0.38^{\text{b}}$			
14 th Day	195.5±0.4	145.0±0.57 ^a	175.6±0.32 ^b	184.7±0.42 ^b	197.2±0.6 ^b			

Values expressed as mean±SEM, n=6 animals in each group. The results were analyzed using One way ANOVA followed by Dunnett's multiple comparison tests. *p<0.05 compared to non-diabetic control, *p<0.05 compared to diabetic control on respective day.

B.C. Koti et al Antihyperglycemic and antihyperlipidemic effect of Rubia cordifolia leaf extract on alloxan-induced diabetes



a. Normal acini and normal cellular population in islets of Langerhans and absence of both damage to islets and hyperplasia in normal control rat pancreas; b. Damaged islets and reduced islet size in diabetic control rat pancreas; c. Restoration of normal cellular population size of islets of Langerhans and absence of islet damage and presence of hyperplasia in glibenclamide treated diabetic rat pancreas; d. Restoration of normal cellular population of normal cellular population of normal cellular population size of islets of Langerhans and absence of islet damage and presence of hyperplasia in glibenclamide treated diabetic rat pancreas; d. Restoration of normal cellular population size of islets of Langerhans and absence of islet damage and presence of hyperplasia in RCAE 400 mg/kg treated diabetic rat pancreas.

performed either in normal or diabetic animal but not together. Since diabetes is a chronic disorder requiring long-term therapy, there is a need to assess the effect of putative hypoglycemic/ antihyperglycemic agents for a longer duration. Grover *et al.*¹⁷, reported that to obtain maximum effect, therapy with a plant product be continued for a longer duration. In addition, if plant extract have a late onset of activity, their effect is likely to be missed in screening studies with shorter duration.

In alloxan-induced diabetic rats, there is partial destruction of β -cells of pancreas due to generation of free radicals. Diabetes is one of the pathological processes known to be related to an unbalanced production of ROS, such as hydroxyl radicals (HO), superoxide anions (O₂⁻) and H₂O₂. Therefore, cells must be protected from this oxidative injury by antioxidant enzymes.

The RCAE were administered for 14 days in both nondiabetic and diabetic groups. The blood glucose level was estimated after 1 h of drug administration during and at the end of the study. The effect of various treatments on total cholesterol, triglyceride, HDL and protein was investigated in normal control and the diabetic groups at the end of the study. GLB was used as standard drug to evaluate the hypoglycemic and antihyperglycemic activity of *Rubia cordifolia*.

In this study, the RCAE did not produce dose-dependent blood glucose reduction in normal and diabetic groups. In normal treated groups, a significant blood glucose reduction was observed up to 10th day, where as in the diabetic groups, significant reduction in blood glucose was maintained up to the 15th day. The extract showed optimum reduction in blood glucose level at 200 mg/kg but at a higher dose (400 mg/kg) it did not show a matching decrease in blood glucose level. Anthraquinones, sterols and saponins have been reported to be present in alcohol extract of *Rubia cordifolia*. The hypoglycemic activity of *Rubia cordifolia* extract may be due to presence of one or more such constituents.

CONCLUSION

In conclusion, our results suggest that the RCAE possess the hypoglycemic effect both in non-diabetic and alloxaninduced diabetic rats. The histopathological report of pancreas of RCAE 400 mg/kg treated rats showed regeneration of pancreatic β -cells. The extract also showed improvement in parameters like body weight and lipid profile. So the hypoglycemic effect of *Rubia cordifolia* might be due to the release of insulin from β -cell of pancreas or might be due to the peripheral utilization of glucose.

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Larvicidal and repellent activities of Ethanolic Extract of *Pongamia Pinnata* Leaves against Mosquitoes

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Mosquitoes are responsible for spread of many diseases than any other group of arthropods. Diseases such as malaria, filariasis, dengue haemorrhagic fever and chikunguinya are real threat to mankind. In the present study ethanolic extract of leaves of *Pongamia pinnata* was evaluated for larvicidal and repellent activities against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus.* The *Pongamia pinnata* leaves were collected and authenticated from Gandhi Krishi Vignan Kendra, Bangalore. The leaves were shade-dried, powdered and extracted using ethanol. The ethanolic extract of *Pongamia pinnata* leaves showed LD₅₀(346.94, 124.20, 359.48 ppm) against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus.* The ethanolic extract of *Pongamia pinnata* leaves provided complete protection time (Mosquito repellency) of 99.96,

¹ 141.35, 144.73 minutes against Aedes aegypti, Anopheles stephensi and Culex quinquefasciatus at higher concentration (1%).

Keywords: Pongamia pinnata, Larvicidal, Repellent, Mosquitoes.

INTRODUCTION

Mosquitoes are well known vectors of several diseases causing pathogens¹. Mosquito-borne diseases, such as malaria, filariasis, dengue haemorrhagic fever (DHF) and chikunguiniya still impose to be major public health problem in the Southeast Asian countries because of their tropical or subtropical climate². *Aedes aegypti* is known to transmit dengue and yellow fever (*Coquillettidia fuscopennata*); malaria being transmitted by *Anopheles species*; and filarial disease through *Culex quinquefasciatus*¹. These diseases not only cause high levels of morbidity and mortality, but also inflict great economic loss and social disruption on developing countries³. Urbanization with changing pattern of lifestyle envisages the proliferation of larval habitats resulting in disease epidemics⁴.

WHO has declared mosquito as "Public enemy number one"⁵. Synthetic insecticides are known to be toxic and adversely affect the environment by contaminating soil, water and air⁶. The high number of breeding places in recent time has resulted in the recrudescence of these diseases. Botanical pesticides are the alternate means which are effective, environment-friendly, and easily biodegradable and also inexpensive⁷.

Some repellents of synthetic origin have limitations such as

RGUHS Journal of Pharmaceutical Sciences Received: 30/01/2011, Modified: 23/02/2011, Accepted: 01/03/2011 skin irritation, unpleasant smell, oily feeling to some users and also potentially toxic. Majority of commercial repellents are prepared by using chemicals like allethrin, N-N-diethylm-toluamide (DEET), dimethyl phthalate (DMP) and N, Ndiethyl mendelic acid amide (DEM)⁸.

In Traditional siddha medicine, *Pongamia pinnata* was used as a mosquito repellent. The present study was an attempt to explore larvicidal and mosquito repellent activities of ethanolic extract of *Pongamia pinnata leaves* against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus*.

MATERIALS AND METHODS

Collection of plants and extraction

Fully developed leaves of *Pongamia pinnata* were collected and authenticated by Dr. Rajanna (Botanist), Department of botany, G.K.V.K, Bangalore, India. The leaves were washed with tap water, shade dried and powdered. The powdered plant material was loaded in soxhlet apparatus and was extracted with ethanol. The extract was subjected to vacuum evaporator to collect the crude extract. Standard stock solutions were prepared by dissolving the residues in the ethanol. These solutions were used for larvicidal and mosquito repellent bioassays.

Larvicidal bioassay

Larvicidal efficacy of ethanolic extract of *Pongamia pinnata* leaves was tested against late 3rd or early 4th instar larvae of *Aedes aegypti, Anopheles stephensi* and *Culex quinquefaciatus*

employing standard WHO procedure⁹. Batches of 25 larvae were exposed to known concentration of test solution (249 ml dechlorinated tap water and 1ml of DMSO dissolved test extract) in glass beakers of 500 ml capacity. Three replicate sets were tested with a final tally of 75 larvae for each concentration. Solutions containing 249 ml tap water and 1ml of DMSO, without plant sample served as controls. No food was provided to the larvae during the test period. Mortality and survival were monitored after 24 h of treatment. The moribund and dead larvae in replicates were combined and expressed as percentage mortality. The larvae were considered as dead or moribund, if they were not responsive to a gentle prodding with a fine needle. Experiments were conducted at 25 ± 2 °C, 12 h light/dark regime. The observed percent mortality was adjusted with the control mortality, using Abott's formula¹⁰ and expressed as corrected mortality. These were subjected to regression analysis of probit mortality on log dosage using computerized log-probit analysis, which provided the lethal dosage of 50, 90, 95 and 99ppm as well as their 95% confidence limit¹¹.

Repellency tests

The ethanolic extract of *Pongamia pinnata* leaves was evaluated for repellent activities against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* using the human – bait technique¹². The plant extract was diluted in ethanol and 0.1, 0.5 and 1% concentrations were prepared. For each test, 10 disease free, laboratory-reared female mosquitoes were placed into separate laboratory cages

(45x38x38 cm)¹³. The mosquito progenies obtained from laboratory colony were maintained in a cloth cage under controlled temperature (28±2°C) and relative humidity range (75-80%)¹⁴. Three volunteers were considered for performing human-bait technique. Before each test, the volunteer's skin was washed with unscented soap and the tested solution was applied from the elbow to the finger tips ¹³. The test was repeated at every 30 min interval. The interval between the application of repellent and the first two consecutive bites occurring within 30 min was considered as protection time against the bites afforded by each of the concentrations of the test repellents¹⁴. In each cage one arm was inserted for one test concentration and the other arm applied with ethanol served as control. The treated and control arms were interchanged regularly to eliminate bias. Each test concentration was repeated three times and in each replicate subject different volunteers to nullify any effect of color of the skin on repellent. Volunteers were asked to follow the testing protocol¹³. The complete protection time (min) for each concentration was calculated¹⁴.

RESULTS

Results of the larvicidal and repellent activities of ethanolic extract of *Pongamia pinnata* leaves against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* are presented in Table : - 1 and 2. The data was recorded, the LD $_{50}$ (50% lethal dose), LD $_{90}$ (90% lethal dose), 95 % Confidence limit and Chi–square value were calculated.

Table1: Larvicidal efficac of Pongamia pinnata leaves against Aedes aeg pti, Anopheles stephensi and Culex quinquefasciatus.							
S.No	Mosquito species	LD ₅₀ (50%	LD (90%	95 % (Confidence	Chi – square		
		lethal dose)	lethal dose)	limit)	value(x ²)		
		(ppm)	(ppm)				
1.	Aedes aegypti	346.9423	950.0453	0.0039 ± 0.0024	7.7784		
2.	Anopheles stephensi	124.2068	309.7531	0.0119 ± 0.0064	4.4232		
3.	Culex quinquefasciatus	359.4834	796.4536	0.0035 ± 0.0024	24. 4758		

ppm – parts per million, Statistical significance:- With increase in concentration, the moratality rate is increasing.

Table 2: Mosquito Repellent activity of Pongamia pinnata against blood starved female Aedes aegypti, Anopheles stephensi and Culex quinquefasciatus.						
Sl.No	Concentration(%)	Complete Protection Time (Min)				
		Aedes Anopheles Culex				
		aegypti	stephensi	quinquefasciatus		
1	Control	1.02	3.7	5.8		
2	0.1	20.38	41.78	40.23		
3	0.5	50.98	74.08	74.23		
4	1	99.96	141.35	144.73		
Statistical significance:- With increase in concentration, the complete protection time is increasing						

DISCUSSION

Previous studies on ethanolic extract of Annona squamosa leaves has shown $LD_{50}(20.70 \text{ ppm})$, $LD_{90}(76.73 \text{ ppm})$ against Aedes albopictus and $LD_{50}(6.96 \text{ ppm})$, LD_{90} (31.80 ppm) against Culex quinquefasciatus¹⁵. The ethanolic extract of Capsicum annum fruits shown $LD_{50}(0.011 \text{ ppm})$, $LD_{90}(0.027 \text{ ppm})$ against Anopheles stephensi¹⁶. The ethanolic extract of Pongamia pinnata leaves in the present study showed that LD_{50} (346.9423 ppm), LD_{90} (950.0453 ppm) against Aedes aegypti, $LD_{50}(124.2068 \text{ ppm})$, LD_{90} (309.7531ppm) against Anopheles stephensi and LD_{50} (359.4834ppm), LD_{90} (796.4536 ppm) against Culex quinquefasciatus.

Earlier studies done on essential oil from leaves of *Ocimum* basilicum showed effective repellency 82.4 ± 0.7 , 75.0 ± 1.2 and 115.3 ± 1.9 mg/mat against *Aedes aegypti, Anopheles* stephensi and *Culex quinquefasciatus*¹⁷. Other studies with petroleum ether extract of *Zanthoxylum limonella* fruits provided protection time of 296 min and 223.5 min against *Aedes albopictus* in mustard oil base and coconut oil base respectively¹⁸. The ethanolic extract of *Pongamia pinnata* leaves provided complete protection time of 99.96, 141.35 and 144.73 mins against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus*.

CONCLUSION

The ethanolic extract of *Pongamia pinnata* leaves showed excellent larvicidal activity against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* and repellency against *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti* respectively. Larvicidal and repellent activities of ethanolic extract of *Pongamia pinnata* leaves in this study against the vectors are encouraging.

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Physicochemical Properties of flour and isolated starch from Jackfruit seeds (*Artocarpus Heterophyllus lam*)

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Jackfruit *(Artocarpus heterophyllus Lam.)* is one the most significant evergreen trees grown in Asia. The objective of this study was to compare physicochemical properties of jackfruit seed flour and starch, starch was isolated from jackfruit seeds and purified. The jackfruit seed flour and starch were subjected to checkout the parameters like organoleptic characteristics, chemical analysis, limit test physico-chemical analysis and micromeritic properties. The chemical composition of seed flour was carbohydrates, polysaccharides, proteins, steroids and amyloses and content of jackfruit seed starch was carbohydrates, polysaccharides, proteins, steroids and amyloses and content of flour was 6.78. The moisture content of starch was more than that of flours. The pH of starch was shows 6.51 and pH of flour was 6.78. The starch was characterized by using scanning electron microscopy method. The shape of the starch was found to be smooth and spherical shaped and size of starch was 6.2mm. Gelatinization and pasting characteristics of starch shows at 65-70°C. Swelling index was 5.96 and 8.03 (g/g) for seed flour and purified starch respectively. Microbial growth was absent in seeds flour and isolated jackfruit starch respectively. Microbial growth was absent in showed good.

Keywords: Jackfruit, Starch, Gelatinization and Pasting characteristics.

INTRODUCTION

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Jackfruit (Artocarpus heterophyllus Lam.) is one the most significant evergreen trees in tropical areas and widely grown in Asia including India. It is a medium-size tree typically reaching 28-80ft in height that is easily accessible by its fruit. The fruit, which borne on the heavy side branch and main branch of the tree, average weight of the fruit is between 3.5 to 10 kg and sometimes the fruit may reach up to 25 kgs of each. Jackfruit has a multiple fruit with a green to yellow-brown outer peel that is composed of hexagonal, bluntly conical thorny that cover a thick. It is a medium-size tree typically reaching 28-80ft in height that is easily accessible by its fruit, the ripe fruit contains well succulent, aromatic and flavorful yellow sweet bulbs and seeds make-up around 8 to 15% of the total fruit weight seeds. The fruit is held together by a central fibrous core. The interior consists of large bulbs along with whitish yellow aril. Each bulb encloses a smooth, oval shaped, brown spermoderm covered by a thin white cotyledon. The seed is 2-3 cm in long and 1-2 cm in diameter. Up to 500 seeds can be found in a single fruit.

Jackfruit is eaten raw or used in curries, soups, chips, juice, jam, jellies and stews and also a common ingredient in fruit salads. The leaves used for animal feeds and white latex are

RGUHS Journal of Pharmaceutical Sciences Received: 31/01/2011, Modified: 23/02/2011, Accepted: 01/03/2011 used as adhesives. The seeds are roasted and eaten like chestnuts, seeds are used in cooked in dishes and its flour used for baking. Jackfruit seeds are fairly rich in starch (Singh et al., 1991). Various parts jackfruit tree were used as medicine and its wood is an important source of timber industries (Roy and Joshi. 1995)

The objective of this study was isolated the starch and some of its physicochemical properties and micromeritic properties were examined from a local origin of jackfruit variety. In this research work we Concluded, that isolated starch can be used as excipients of pharmaceutical dosage mainly in tablet preparation as binding agent.

Botanical description:

Preferred scientific name: Artocarpus heterophyllus Lam.

Family: Moraceae (mulberry family) Non-preferred scientific names: Artocarpus brasiliensis Gomez Artocarpus heterophylla Lam. Artocarpus maxima Blanco Artocarpus philippinensis Lam. Polyphema jaca Lour. Soccus arboreus major Rumph.

Artocarpus integer (Thunb.) Merr and its synonym A. integrifolia

Fig.1: Photographs of Jackfruit and Jackfruit seeds

L. f. are a different species (champedak), and these names have often mistakenly been used as synonyms for *A.heterophyllus*.

Regional names:

Halasu or Halasina Hannu – Kannada

Kanthal, Kathal, Kathar, Panos - Hindi

Murasabalam, Pala, Panasa-Tamil

Panasa, Verupanasa-Telugu

Chakka, Pilavu-Malayalam

MATERIALS AND METHODS

The jackfruit seeds were obtained from local market in India. All reagents and chemicals used in the experiment were of analytical grade and purchased from their respective commercial sources.

Isolation and purification of starch from the jack fruit seeds: The collection seeds were washed with running water to remove their impurities. The outer brown layers were removed by soaking jackfruit seeds in 0.5% NaOH solution for 30 ml and rinsing with distilled water and again washed with running water. The washed seeds were cut into small pieces and it was grinded into a fine paste. The Paste was added with required amount of water (5 l) and kept for 12 h for settling. Purification is effected by stirring with water and re- suspending several times. The fine settled product dried in a tray drier at 30-40°C until the moisture content was less than 13%, than dried product passed through suitable sieve. This dried product was stored in desiccators and to carried out the organoleptic characteristics, starch conformity test physicochemical properties and its micromeritic properties.

Organoleptic evaluation of starch: The organoleptic evaluation of the isolated starch such as color, odor and taste were determined after isolation and drying of the starch.

Chemical analysis:

Test for Carbohydrates

Molish test: Mix 2ml of sample solution and add five drops

of molish reagent in a test tube. Add gently MI of of concentrated sulphuric acid at the sides of the test tube.

Test for reducing and non reducing sugars: Benedicts test: To 5ml of the regent add drops of sugar solution mix and keep in the boiling water for min.

Barfoed test: To 5ml of reagent, add about 0.5ml of sample solution. Keep it in boiling for 5 m.

Test for Polysaccharides:

1. Iodine Test: To about 2ml of starch solution add 2 to 3 drops N/50 iodine.

2. Add 1ml of 5% NaOH to 2ml starch to make it alkaline. No blue color on acidification gives blue color. It confirms the presence of starch.

3. On treatment with boiling water, a cloudy viscous fluid was obtained. This also confirms the presence of starch.

Test for Proteins:

Biuret test: To 3ml of the sample solution add an equal volume of 5% NaOH and 3 to 4 drops of 1% copper sulphate.

Test for Steroids: To about 2ml of a solution of cholesterol in chloroform in a dry test tube, add 2ml of acetic anhydride and 2 to 3 drops of concentrated sulphuric acid. Mix and stand for few minutes.

Test for Amylose: 20 mg of isolated starch sample was taken and 10 ml of 0.5 N KOH was added to it. The suspension was thoroughly mixed. The dispersed sample was transferred to a 100 ml volumetric flask and diluted to the mark with distilled water. 10 ml of test starch solution was pipette into a 50ml volumetric flask and 5 ml of 0.1 N HCl was added followed by 0.5 ml of iodine reagent. The volume was diluted to 50ml and the absorbance was measured at 625 nm.

Limit test:

Test for Chlorides:standard turbidity: for standard solution 1ml of 0.05845 percent w/v solution of sodium chloride in nessler cylinder add 10ml of nitric acid, make up the volume upto 50ml with water, add 1ml of silver nitrate solution. Stir immediately with a glass rod and keep it for few minutes.

Sample turbidity: Dissolve the specified quantity of sample substance in distilled water. Add 1ml of dilute nitric acid in the nessler cylinder. Then make up the volume up to 50ml with water and add 1ml silver nitrate. Stir well and keep aside for 5 m.

Test for Sulphates: standard turbidity: for standard

opalescence 1ml of 0.1089 percent w/v solution of potassium sulphate and add 2ml of dilute hydrochloric acid in nessler cylinder. Add 45ml of water and add 5ml barium chloride reagent. Stir well and keep it for 5 m.

Sample turbidity: To the given add distilled water and mixted with 2ml of dilute hydrochloric acid and distilled water upto 45ml and 5ml of barium chloride. Stir well and keep it for 5 m.

Test for Iron: standard turbidity: dilute 2ml of standard iron solution with 40 ml of distilled water in the nessler cylinder. Add 2ml of a 2% w/v iron-free citric and 0.1ml of thioglycollic acid and mix. Make alkaline with iron free ammonia solution. Dilute the level to 50 ml and allow stand for few minutes.

Test turbidity: to the given sample add 40ml of water or used10 ml of the as prescribed in the monograph and transfer into the nesselers cylinder. Add 2mL of 20% /v solution of iron free citric acid and 0.1ml of thioglycollic acid and mix. Make alkaline with iron free ammonia. Dilute to 50 ml of water and allow standing.

Physico-chemical analysis:

Moisture content determination: Two gram of each test samples (dry base) was dried in a conventional oven at 105°C to the constant weight (approximately 3 h).

Determination of pH: The pH was measured by using a digital pH meter. The results are shown in table No 2.

Scanning Electromicroscopy Analysis: The size and shape of the isolated starch was determined by scanning electron microscopy. The results are shown on figure No 2.

Viscosity: Flow property of a simple liquid is expressed in terms of viscosity. Viscosity is an index of resistance of a liquid to flow. The higher the viscosity of a liquid, the greater is the resistance to flow. The viscosity was measured by using a brook field viscometer. The results are shown on table No 2.

Gelatinization and Pasting characteristics of starch: Samples of starch powder were moistened with water and loaded into capillary tube by means of intrusion. The temperature of gelling and the time from swelling to full gelatinization were measured with a melting point apparatus.

The Pasting characteristic of sample starch powder was observed by suspending it in 10ml of distilled water and heating with stirring on a water bath. The results are shown on table No 2.

Swelling index: one gram of powdered mucilage was treated with 25ml of water in a graduated cylinder shaken for every 10 m for 1 h and allowed to stand as specified. The results are shown on table No 2.

Swelling Index = Weight of wet mass / Weight of dry powder

Water absorption Index: One gram of sample was suspended in 10ml of distilled water at 30°C in a centrifuge tube, stirred for 30 m intermittently and then centrifuged at 3000 rpm for 10 m. the supernatant was decanted and the weight of the gel formed was recorded. The water absorption Index was than calculated as gel weight per gram dry sample.

Water absorption Index = Bound water (g) / Weight of sample (g) X 100

Total microbial load of the isolated Jack fruit starch: The total microbial load is an important parameter which decides the suitability of a substance for use as an excipient in pharmaceutical dosage form. According to many pharmacopoeias, for synthetic and semi synthetic substances, the total aerobic count should not be more than 100 colonies forming unit (cfu) per total fungal count (including yeast and molds) should not exceed 50 cfu/g. in case of excipients from natural origin the total aerobic count should not be more than 1000 cfu/g and total fungal count should not exceed 100 cfu/g. The results are shown on table 2.

Micromeritic properties:

Angle of repose: Flow properties of seeds flour and isolated starch were studied by measuring the angle of repose by employing fixed funnel standing method.

A glass filling funnel is held in place with a clamp on the ring support over a glass plate. Seeds flour and isolated starch were weighted passed through the funnel, which was kept at a height 'h' from the horizontal surface. The passed starch powder through the funnel, its formed a pile of a height 'H' above the horizontal surface and the pile was measured and the angle of repose was determined by using the formula.

> Angle of repose $(\theta) = \tan^{-1}(H/R)$ H = Height of the pile R= Radius of the pile.

Bulk density and Tapped density: Bulk and tapped densities were measured by using 100 ml of graduated cylinder. The sample poured in cylinder was tapped mechanically for 100 times, then tapped volume was noted down and bulk density and tapped density were calculated.

Each experiment for micromeritic properties was performed in triplicate manner.

Carr's index: Compressibility index (Ci) or Carr's index value of seeds flour and isolated starch was computed according to the following equation:

Carr's index = (Tapped density – Bulk density) / Tapped density \mathbf{X} 100

RESULTS

The starch was isolated from the jackfruit seeds and purified by reported methods. The yield was found to be 25% w/w. The flour was prepared by grinding jackfruit seeds without removing thin brown spermoderm. The flour and starch was subjected to characterization such as organoleptic evaluation, chemical test, limit test, physico - chemical test and micromeritics showed in tables.

DISCUSSION

Organoleptic evaluation: The jackfruit starch was white in color compared with the flour. Color of the jackfruit seed flours was light yellow. The color of flour can be influence by contenting of variety of raw material. Odor of jackfruit seed starch and flour were odorless and also had mucilaginous taste.

Chemical Analysis: Chemical composition of isolated jackfruit starch and seed flours was significantly different

Molish test was formed a purple color ring at the interface of the two layers. It indicates the presence of carbohydrates. Benedicts test for seeds flour and purified starch was producing red precipitate; it indicates the presence of reducing sugar. Barfoed's test for seeds flour was producing precipitate but purified starch wasn't producing precipitate; it indicates the absence of reducing disaccharides.





Test for Polysaccharides, Starch and flour sample was formed blue color from starch iodine complex. It confirms the presence of starch. Starch to make it alkaline. No blue color on acidification gives blue color. It confirms the presence of starch. On treatment with boiling water, a cloudy viscous fluid was obtained. This also confirms the presence of starch

Biuret test for seed flour, the was gives purple color or pinkish purple color. It indicates the presence of proteins,

	Table 1: Chemical Analysis of seed flour and isolated purified Starch.					
S.N	Chemical tests		Flour	Flour.		
1	Test For Carbohydrates Molish test		Presence of carbohydrates	Presence of carbohydrates		
		Benetict's test	Presence of reducing sugar	Presence of reducing sugar		
		Barfoed's test	Presence of reducing disaccharides	Absence of reducing disaccharides		
2	Test for Polysaccharides	Iodine test	Confirms the presence of starch.	Confirms the presence of starch.		
3	Test for Proteins	Biuret test	Presence of proteins	Absence of proteins		
4	Test for Steroids	Libermann	Presence of steroids burchard test	Absence of steroids.		
5	Test for Amylose		Presence of amylose	Presence of amylose		

Table 2: Physico-chemical analysis of seed flour and isolated purified Starch.						
Parameters	Flour	Starch				
Moisture Content	Less	More				
Gelatinization temperature	$65-70^{\circ}C$	$65-70^{\circ}\mathrm{C}$				
pН	6.78	6.51				
Viscosity:						
10% std	13000cps	9600 cps				
10% sample	13000cps	5400cps				
Swelling index (g/g)	5.96	8.03				
Water absorption Index	Less	More				
Microbial growth	Absence	Absence				

Table 3: Micromeritic properties of jackfruit seeds flour and isolated purified Starch .						
Parameters Flour Starch						
Angle of repose	26°55 "	29°45 "				
Bulk density	0.487	0.398				
Tapped density	0.578	0.477				
Carr's index 15.74 16.56						

but for starch wasn't gives purple color or pinkish purple color. It indicates the absence of proteins

Liebermann burchard test for flour, sample was producing emerald green color. It indicates the presence of cholesterol. Isolated starch sample wasn't produce emerald green color. It indicates the absence of cholesterol. Amylase content of isolated jackfruit seed starch was highest, compared to the seed flour. Limit Test for Chlorides, Sulphates and Iron was passing.

Physico-chemical Analysis: The moisture content of starch from jackfruit seed was more than that of flours. The pH of starch was shows 6.51 and pH of flour was 6.78. The isolated starch was analyzed for its size of and morphological characters by scanning electron microscopy method. The size of the starch was found to be 6.2mm. The surface of the starch was smooth and spherical shape.

Gelatinization and Pasting characteristics of starch shows at 65-70°C. Seeds flour was containing more viscosity compares to purified starch. Swelling index was 5.96 and 8.03 (g/g) for seed flour and purified starch respectively. Water absorption index of flour and starch were indicated good ability to bind water. Microbial growth was absent in seeds flour and isolated jackfruit starch respectively. Percentage yield was 22 % (+/-3%).

Micromeritic properties: Angle of repose of seeds flour and isolated purified starch showed excellent flowability. Bulk and tapped densities showed good packability of starch. Carr's index of seeds flour had 15.74 and isolated purified starch showed 16.56, lowest Carr's index of both flour and starch indicating excellent compressibility.

CONCLUSION

This study suggests that jackfruit seed flour and purified starch has a potential for food formulation because of its containing amylase and carbohydrate contents. The jackfruit seed starch had a narrow gelatinization temperature and required less energy for gelatinization, it helpful for make a starch paste it's used as a granulating agent. Starch of jackfruit seeds showed a greater stability to thermal during making starch paste. This starch can be used as excipients of pharmaceutical dosage in tablet preparation, thus it could be more useful as a binding agent. Angle of repose of starch showed excellent flowability, Bulk and tapped densities showed good packability of starch and Carr's index indicating excellent compressibility. So purified starch of jackfruit seeds could be substituted for it as a excipients when the need arises.

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Evaluation of Anticonvulsant Activity of Carissa spinarum Root Extract

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In the present study the ethanol extract of the roots of *C. spinarum* (100, 200 and 400 mg/kg, p.o.) was studied for its anticonvulsant effect on maximal electroshock, pentylenetetrazole and picrotoxin-induced seizures in mice. The latency of tonic convulsion and the number of animals protected from tonic convulsion were noted. The ERCS at dose levels of 100, 200 and 400 mg/kg significantly reduced the latency of tonic seizures and at 200 and 400 mg/kg, respectively protected 25 and 62.5 % of the mice used from tonic seizures induced by maximal electroshock. The ERCS in the doses of 200 and 400 mg/kg respectively protected 50 and 62.5 % of animals used and significantly delayed pentylenetetrazole-induced tonic seizures. Similarly, the same doses of ERCS significantly delayed the onset of tonic seizures produced by picrotoxin. The data suggest that the ethanol extract of the roots of *C. spinarum* may possess significant anticonvulsant activity via non-specific mechanisms, since it has been shown to delay the latency of seizures produced by the convulsive models affecting electrical discharge in the brain, gabaergic system and glutaminergic systems.

Keywords: Anticonvulsant, Carissa spinarum, Electro shock, Seizures

INTRODUCTION

Epilepsy is a common neurological disorder and a collective term given to a group of syndromes that involve spontaneous, intermittent, abnormal electrical activity in the brain which affect up to 5% of the world population in their lifetime. The current therapy of epilepsy with modern antiepileptic drugs is associated with side effects, doserelated and chronic toxicity, teratogenic effects and approximately 30% of the patients continue to have seizures with current antiepileptic drugs therapy. Traditional systems of medicine are popular in developing countries and up to 80% of the population relies on traditional medicines or folk remedies for their primary health care need. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects¹. Therefore, the need for more effective and less toxic antiepileptic drugs still exists.

Carissa spinarum Linn. (*Carissa opaca* Stapf ex Haines, Family: Apocynaceae) is a thorny, evergreen shrub, widely distributed throughout the drier, sandy and rocky soils of India, Ceylon, Myanmar and Thailand². The roots of this plant have long been prescribed in the indigenous system of medicine as purgative, for the treatment of rheumatism, cleaning worm infested wounds of animals and in snake

RGUHS Journal of Pharmaceutical Sciences Received: 31/01/2011, Modified: 28/02/2011, Accepted: 07/03/2011 bite². Previous phytochemical investigations revealed the presence of caffeic acid³, ursolic acid, naringin⁴, various cardiac glycosides⁵, germacrane sesquiterpene and lignans⁶. Earlier studies have shown that the extract of the plant possesses cardiotonic⁷, antibacterial⁴, potent antioxidant activity⁶. Moreover the plant is also known to show CNS depressant activity⁸. In the Western Ghats region of India, the decoctions of the roots of this plant are used as effective remedies in the management and/or control of convulsions and epilepsy. Since no systemic study has been reported in the literature to explore the folklore claim, the present study was undertaken to evaluate possible actions of *C. spinarum* root extract on convulsive and epileptic disorders in mice using electrically and chemically-induced seizures.

MATERIALS AND METHODS

Plant Material and Preparation of the Extract: The roots of *C. spinarum* were collected from Sirsi, Uttara Kannada District, Karnataka, India during May 2007. It was authenticated by Dr. Gopalakrishna Bhat, Department of Botany, Poorna Prajna College, Udupi, Karnataka, India. A voucher specimen no. 105b is deposited in the herbarium of our institute. Fresh roots were collected and dried by means of shade drying. The shade dried roots of the plant (500 g) were soaked in 1.5 L of 95% ethyl alcohol and extracted in the cold for 4 days with occasional shaking. After 4 days the ethanol layer was decanted off. The process was repeated for 4 times. The solvent from the total extract was

filtered, the concentrate was evaporated to dryness under reduced pressure and low temperature (40°C) on a rotary evaporator to give the ethanolic extract (13% w/w yield), which was stored at 4°C until use. Suspension of the extract was prepared in 1% Tween-80 and used to assess pharmacological activities.

Experimental Animals: Healthy Swiss mice of either sex, weighing about 25-30 g and Wistar rats of either sex, weighing about 150-200 g were used in experiments. Animals were housed in polypropylene cages maintained under standard conditions (12 hours light / dark cycle; 25 ± 3 °C, 45-65 % humidity) and had free access to standard rat feed (Kamadenu Agencies, Bangalore, India) and water *ad libitum*. All the animals were acclimatized to laboratory conditions for a week before commencement of experiment. All experimental protocols were reviewed and accepted by the Institutional Animal Ethical Committee (IAEC) prior to the initiation of the experiment.

Drugs and Chemicals: Pentylenetetrazole (PTZ; Sigma Chemical Co. USA), picrotoxin (PC; Sigma Chemical Co. USA), phenobarbitone (PHNB; Phenetone, Cipla, Mumbai) and 5,5-diphenylhydantoin sodium salt (Phenytoin, PHNY; Sigma Chemical Co. USA) were all dissolved in physiological saline. Diazepam (DZP; Calmpose, Ranbaxy Laboratories, Gurgoan, India) was also suspended in a minimum amount of polyethylene glycol 400 (Ranbaxy Laboratories, Gurgoan, India) and adjusted to an appropriate volume with physiological saline. The pretreatment dose and the times following the administration of either pentylenetetrazole (90 mg/kg, i.p.) and picrotoxin (10 mg/kg, i.p.) were ethanolic extract of the roots of *C. spinarum* (100, 200 and 400 mg/kg, p.o., 30 min), diazepam (0.5 mg/kg, i.p., 20 min), phenobarbitone (10 mg/kg, i.p., 10 min) and phenytoin (25 mg/kg, i.p., 20 min).

Phytochemical Screening: Freshly prepared ethanol extract of the roots of *C. spinarum* (ERCS) was subjected to phytochemical screening tests for the detection of various constituents⁹.

Acute Toxicity Study: Acute toxicity study of ethanolic extract of the roots of *C. spinarum* (ERCS) was determined in male Wistar albino rats (150-180 g) according to OECD guidelines No. 425¹⁰. The animals were fasted overnight and the ethanolic extract was administered orally with a starting dose of 2000 mg/kg to a group containing three animals. Animals were observed continuously for first 3 h and monitored for 14 days for mortality and general behavior of animals, signs of discomfort and nervous manifestations.

Electrically-Induced Seizures: In electrically induced

seizures the maximal electroshock (MES) method described previously by Swinyard was employed¹¹. In brief, tonic convulsions of the hind extremities of mice were induced by passing alternating electrical current of 50 Hz and 150 mA for 0.2 sec through corneal electrodes. The number of animals protected from hind limb tonic extension seizure (HLTE) and the time spent in this position were determined in each dose group. Experiments were repeated following the pretreatment of animals with ethanol extract of the roots or *C. spinarum*, diazepam, phenobarbitone and phenytoin or control vehicle prior to the induction convulsion.

Chemically-Induced Seizures: Modified method of Vellucci and Webster was used to assess the anticonvulsant effect of the ethanol extract of the roots of C. $spinarum^{12}$. Seizures were induced in mice with standard convulsing agents, pentylenetetrazole (PTZ) and picrotoxin (PC), and the animals were observed for 30 min for tonic convulsion episode. Hind limb extension was taken as tonic convulsion. The onset of tonic convulsion and the number of animals convulsing or not convulsing within the observation period were noted. Experiments were repeated following the pretreatment of animals with ethanol extract of the roots of C. spinarum, diazepam, phenobarbitone and phenytoin or control vehicle prior to the administration of any of the convulsing agents used. The ability of the plant extract to prevent or delay the onset of the hind limb extension exhibited by the animals was taken as an indication of anticonvulsant activity.

Statistical Analysis: The results on the onset of seizures were analyzed using the paired Student's't'-test while the proportion of animals that exhibited tonic seizures was analyzed using Chi-square test. A p value of <0.05 was considered as statistically significant.

RESULTS

Phytochemical Screening: Phytochemical screening of the ERCS showed that the crude extract contained flavonoids, saponins, cardiac glycosides, triterpenoids, phenolic compounds and tannins.

Acute Toxicity Study: There was no mortality amongst the dosed group of animals and they did not show any toxicity or behavioral changes at a dose level of 2000 mg/kg. This finding probably suggests that the ethanol extract of the roots of *C. spinarum* is relatively safe in or non-toxic to mice and 100, 200 and 400 mg/kg doses were used for this study.

Anticonvulsant Properties Assessment: Maximal electroshock produced hind limb tonic extension seizures (HLTE) in all the animals used. The vehicle treated mice

showed tonic hind limb extension for a duration of 16.51 ± 0.23 sec. ERCS (100 mg/kg) significantly reduced the latency, but did not alter the incidence of seizures elicited by maximal electroshock to any significant extent. ERCS in the doses of 200 and 400 mg/kg, respectively protected 25 and 62.5% of mice and significantly reduced the duration of the seizures. The standard antiepileptic drugs, diazepam protected 62.5% of mice against seizures and significantly reduced the duration of the seizures is phenobarbitone significantly protected 87.5% of mice and significantly reduced the duration of the seizures. However, phenytoin profoundly antagonized the MES-induced tonic seizures in all the animals used (Table 1).

Pentylenetetrazole produced tonic seizures in all the animals used. A dose of 100 mg/kg of ERCS protected 25% of animals against seizures and did not affect the onset of the seizures to any significant extent. ERCS in the doses of 200 and 400 mg/kg, respectively protected 50 and 62.5% of mice against seizures, and significantly delayed the latency of the seizures. The standard antiepileptic drugs, diazepam and phenobarbitone profoundly antagonized the tonic seizures. Phenytoin neither affected the onset nor the incidence of convulsion to any significant extent (Table 1). The control vehicle did not alter PTZ-induced seizures to any extent (results not shown).

Picrotoxin produced tonic seizures in all the animals used. ERCS (100 mg/kg) did not affect the incidence or the latency of seizures. A dose of 200 and 400 mg/kg of ERCS did not affect the incidence, but significantly prolonged the latency of seizures. The standard antiepileptic drugs, diazepam significantly reduced the number of animals convulsing and significantly prolonged the latency of seizures, where as phenobarbitone did not alter the incidence, but profoundly delayed the onset of seizures. Phenytoin neither affected the onset nor the incidence of convulsion to any significant extent (Table 1). The control vehicle did not alter picrotoxin-induced seizures to any extent (results not shown).

Table No.1 - Effect of ethanol extract of the roots of <i>C. spinarum</i> (ERCS) on (A) maximal electroshock (MES), (B) pentylenetetrazole (PTZ) and (C) picrotoxin (PC) - induced seizures in mice							
Dose (mg/kg)				No. of animals	% animals	Latency of HLTE	
-	-	PHNB -	-	А	8/8	0	16.51 ± 0.23
				В	8/8	0	6.78 ± 0.79
				\mathbf{C}	8/8	0	13.99 ± 0.54
100	-	-	-	А	8/8	0	$12.30 \pm 0.14^{\circ}$
				В	6/8	25	9.06 ± 1.27
				\mathbf{C}	8/8	0	15.11 ± 0.61
200	-	-	-	А	6/8	25	9.91 ± 0.20^{a}
				В	4/8	50	$13.09 \pm 1.65^{\circ}$
				\mathbf{C}	8/8	0	$16.57 \pm 0.99^{\circ}$
400	-	-	-	А	3/8	62.5	$6.31 \pm 0.19^{\mathrm{b}}$
				В	3/8	62.5	$15.80 \pm 1.49^{\mathrm{b}}$
				\mathbf{C}	8/8	0	$18.89 \pm 1.57^{\circ}$
-	0.5	-	-	А	3/8	62.5	$5.01 \pm 0.11^{\text{b}}$
				В	0/8 [°]	100	-
				\mathbf{C}	1/8 [°]	87.5	$21.95 \pm 1.78^{\mathrm{b}}$
-	-	10	-	А	1/8 [°]	87.5	4.17 ± 0.15^{b}
				В	0/8	100	-
				С	8/8	0	$23.16 \pm 1.51^{\mathrm{b}}$
-	-	-	25	Α	0/8 ^d	100	-
				В	8/8	0	6.91 ± 0.29
				С	8/8	0	14.07 ± 1.18
^a p < 0.05, ^b p < 0.01, vs. tween-80 treated group (0.25 ml, p.o.); Student's t-test., ^c p < 0.01, ^d p < 0.001 vs. tween-80 treated group (0.25 ml, p.o.); Chi-square test., DZP- Diazepam; PHNB- Phenobarbitone; PHNY- Phenvtoin; HLTE-							

Hind limb tonic extension seizure.

DISCUSSION

GABA is the major inhibitory neurotransmitter in the brain where as glutamic acid is an excitatory neurotransmitter in the brain. The inhibition of GABA neurotransmitter and the enhancement of the action of glutamic acid have been shown to be the underlying factors in epilepsy¹³. Our study shows that the ethanol extract of the roots of *C. spinarum* protected some of the animals against seizures induced by maximal electroshock, pentylenetetrazole and also delayed the latency of the seizures.

In the present study maximal electroshock produced seizures in all the animals used. It has often been stated that antiepileptic drugs that block MES-induced tonic extension act by blocking seizure spread. Moreover, drugs that inhibit voltage-dependent Na+ channels, such as phenytoin can prevent MES-induced tonic extension¹⁴. However, phenobarbitone is as effective against electrically induced convulsion as it is against pentylenetetrazole-induced convulsions in mice and phenobarbitone is known to reduce the electrical activity of neurons within a chemically induced epileptic focus in the cortex, where as diazepam does not suppress the focal activity but prevents it from spreading^{15, 16}. Though, diazepam had anticonvulsant effect on both PTZ-induced seizures and maximal electroshockinduced seizures, in which diazepam effect for the former (100% prevention) is better than the latter (50% prevention). This is consistent with the report that benzodiazepine (BDZ) agonists such as diazepam, etc. are more potent in the prevention of PTZ-induced seizures than in that of MESinduced tonic seizures¹⁷.

In the present study, pentylenetetrazole was shown to induce seizures in all the mice used. Pentylenetetrazole may be producing seizures by inhibiting gabaergic mechanisms¹⁸. Standard antiepileptic drugs diazepam and phenobarbitone are thought to produce its effects by enhancing GABA-mediated inhibition in the brain. It is therefore possible that the anticonvulsant effects shown in this study by the said drug against seizures produced by PTZ might be due to the activation of GABA neurotransmission. Since ethanol extract of the roots of *C. spinarum* similarly antagonized seizures elicited by pentylenetetrazole in mice, it is probable therefore that it may also be exerting its anticonvulsant effects by affecting gabaergic mechanisms.

In the same study, picrotoxin also produced seizures in all the mice used. Picrotoxin has been shown to elicit seizures, by antagonizing the effect of GABA by blocking the chloride channels linked to GABA_A-receptor¹³. In this study, diazepam and phenobarbitone were shown to antagonize

the effect of picrotoxin and ethanol extract of *C. spinarum* was also shown to delay the latency of picrotoxin–induced seizures, suggesting that ethanol extract of the roots of *C. spinarum* may be affecting gabaergic mechanisms, probably by opening the chloride channels associated with GABA-receptors.

Triterpenic steroids and triterpenoidal saponins are reported to possess anticonvulsant activity in some experimental seizure models like MES, PTZ^{19, 20}. Some monoterpenes, flavonoids also have protective effects against PTZ and picrotoxin-induced convulsions^{21, 22}. It is worthwhile to isolate the bioactive principles, which are responsible for these activities, which is in progress in our laboratory. These findings justify traditional use of this plant in the control and /or treatment of convulsions, epilepsy and validate its claim of being used for the said purpose in folklore medicine.

CONCLUSION

It can be concluded that the data obtained in the present study suggest that the ethanol extract of the roots of *C. spinarum* may said to be possess significant anticonvulsant activity via non-specific mechanisms, since it has been shown to delay the latency of seizures produced by the convulsive models which affecting electrical discharge in the brain, gabaergic system and glutaminergic systems and also which may be of potential benefit for the management, control and /or treatment of convulsions and epileptic disorders.

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S nthesis and Pharmacological evaluation of Schiff's and Mannich bases of Indole Derivatives

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Indole-3-carbaldehyde was treated with hydrazine hydrate to form hydrazones of indole. The synthesized derivatives were further treated with various aromatic aldehydes to afford Schiff's bases. On the other hand, Mannich bases of indole derivatives were synthesized by reacting derivatives of indole with various aromatic and heterocyclic amines in the presence of formaldehyde and dimethyl formamide. The newly synthesized compounds were characterized by FT-IR, ¹H NMR & MASS spectra.

- Schiff's bases of indole derivates were evaluated for antibacterial and antifungal activity using cup-plate method. Some of the newly synthesized Schiff's bases showed good antibacterial and antifungal activity.
- Synthesized Mannich bases of indole derivatives were investigated for their anti-inflammatory activity using carrageenan
- induced paw oedema method in rat and analgesic activity using acetic acid induced writhing method in mice. Some of
 Mannich bases of indole derivatives were found to possess good anti-inflammatory and analgesic activity.

Keywords: Indole, Schiff's bases, Mannich bases, antibacterial activity, antifungal activity,

INTRODUCTION

One of the most frequently encountered heterocyclic compounds in synthetic organic chemistry is indole derivatives, as they are found to be potent pharmacophores. Indole derivatives have displayed versatile pharmacological properties such as analgesic¹⁻⁵, anti-inflammatory⁶⁻¹², anticonvulsant¹⁰, antimicrobial¹³⁻¹⁷, antibacterial¹⁸⁻²¹, antifungal²²⁻²⁴, antiviral²³, anticancer²⁵⁻³⁰ activities.

Schiff's bases are important heterocyclic molecules. Many researchers studied the synthesis, characterization and structure-activity relationship (SAR) of Schiff's bases and they are known for their antibacterial and antifungal³¹⁻³⁶, anti-HIV³¹, anti-inflammatory³⁴⁻³⁶, analgesic,³⁶ antitumor,³⁶ antimalarial,³⁶ antidyslipidemic³⁷ activity.

Mannich bases were found to possess potent activities such as anti-inflammatory³⁸, antibacterial and antifungal^{17, 39-41}, antiviral¹⁷, antifilarial³⁸, anticancer, ⁴¹ antiparkinsons, ⁴¹ and anti-HIV⁴¹ drugs. They are also used in polymer industry as paints and surface active agents. Mannich bases demonstrated anticonvulsant^{38,42} activities.

Encouraged by the above observations we have planned to synthesize Schiff's and Mannich bases of indole derivatives.

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MATERIALS AND METHODS

All FT-IR spectra were recorded in Tensor 27 spectrophotometer, Bruker optik (Germany) using ATR method. All ¹H NMR spectra were recorded in Bruker spectrophotometer AMX-400 (400 MHz), Bruker optik (Germany) in CD₃OD using TMS as an internal standard. Mass spectra were recorded using a Joel-D-300 Mass spectrophotometer (70ev), SHIMADZU (Japan) by LCMS-2010A. All melting points were determined in open capillaries using Thermonik precision apparatus (Model-C-PMP-2, Mumbai, India) and are uncorrected. All TLC were performed on precoated TLC plates (Silica Gel 60; F254: Merck, Germany) and visualized under UV light. Animal experimentation was approved by IAEC of Acharya and B. M Reddy college of Pharmacy with ref no: ABMR: EST: IAEC-59-1: 08-09 dated 27-09-2008.

Synthesis of 1H -indole-3-carbaldehyde hydrazone [A]

Equimolar quantities of hydrazine hydrate (1 ml, 0.002 mol) and indole-3-carbaldehyde (1 g, 0.002 mol) were dissolved in 50 ml of absolute ethanol. The mixture was refluxed for 3 h. The progress of the reaction was monitored by TLC. The excess of solvent was removed under reduced pressure. The obtained product was recrystallized with ethanol.

Yield-62%, m.p-156°C, FT-IR 3183cm⁻¹ (NH stretch), 3057cm⁻¹ (Ar. C-H stretch), 2884cm⁻¹ Aliphatic C-H stretch),

1577cm⁻¹ (C=C), 1616cm⁻¹ (C=N), Molecular formula- $C_{9}H_{9}N_{3}.$

General procedure for the synthesis of 1H -indolo-3-carbaldehyde [substituted methylene] hydrazine [A1-A7]

The mixture of 1H-indole-3-carbaldehyde hydrazone (0.43 g, 0.001 mol) and substituted benzaldehyde (0.001 mol) in absolute alcohol (10 ml) was refluxed for 3 h. After TLC monitoring, reaction mixture was poured in crushed ice. The product formed was filtered, washed with cold water and recrystallized from ethanol.

Preparation of 1H-indole-3-carbaldehyde [(1E)-(4-nitrophenyl)methylene]hydrazone (A1)

The mixture of 1H-indole-3-carbaldehyde hydrazone (0.43 g, 0.001 mol) and p-nitro benzaldehyde (0.001 mol) in absolute alcohol (10 ml) was refluxed for 3 h. After TLC monitoring, reaction mixture was poured in crushed ice. The product formed was filtered, washed with cold water and recrystallized from ethanol.

Preparation of 1H-indole-3-carbaldehyde (1E)-(3nitrophenyl)methylene]hydrazone (A2)

The mixture of 1H-indole-3-carbaldehyde hydrazone (0.43 g, 0.001 mol) and m-nitro benzaldehyde (0.001 mol) in absolute alcohol (10 ml) was refluxed for 3 h. After TLC monitoring, reaction mixture was poured in crushed ice. The product formed was filtered, washed with cold water and recrystallized from ethanol.

Preparation of 1H-indole-3-carbaldehyde (1E)-(2nitrophenyl) methylene]hydrazone (A3)

The mixture of 1H-indole-3-carbaldehyde hydrazone (0.43 g, 0.001 mol) and o-nitro benzaldehyde (0.001 mol) in absolute alcohol (10 ml) was refluxed for 3 h. After TLC monitoring, reaction mixture was poured in crushed ice. The product formed was filtered, washed with cold water and recrystallized from ethanol.

Preparation of 1H-indole-3-carbaldehyde (1E)-(4chlorophenyl) methylene]hydrazone (A4)

The mixture of 1H-indole-3-carbaldehyde hydrazone (0.43 g, 0.001 mol) and p-chloro benzaldehyde (0.001 mol) in absolute alcohol (10 ml) was refluxed for 3 h. After TLC monitoring, reaction mixture was poured in crushed ice. The product formed was filtered, washed with cold water and recrystallized from ethanol.

Preparation of 1H-indole-3-carbaldehyde {(1E)-[4-(dimethylamino)phenyl] methylene}hydrazone (A5)

The mixture of 1H-indole-3-carbaldehyde hydrazone (0.43 g, 0.001 mol) and p-dimethy amino benzaldehyde (0.001 mol) in absolute alcohol (10 ml) was refluxed for 3 h. After TLC monitoring, reaction mixture was poured in crushed ice. The product formed was filtered, washed with cold water and recrystallized from ethanol.

Preparation of 1H-indole-3-carbaldehyde [(1E)-2furylmethylene]hydrazone (A6)

The mixture of 1H-indole-3-carbaldehyde hydrazone (0.43 g, 0.001 mol) and furfuraldehyde (0.001 mol) in absolute alcohol (10 ml) was refluxed for 3 h. After TLC monitoring, reaction mixture was poured in crushed ice. The product formed was filtered, washed with cold water and recrystallized from ethanol.

Yield-67%, m.p-128°C, FT-IR 3198cm⁻¹ (NH stretch), 3054cm⁻¹ (Ar. C-H stretch), 2878cm⁻¹ (Aliphatic C-H stretch), 1577cm⁻¹ (C=C), 1616cm⁻¹ (C=N), 1356cm⁻¹ (ring C-O stretch), Molecular formula- $C_{14}H_{11}N_3$.

Preparation of 1H-indole-3-carbaldehyde [(1E, 2E)-3-phenylprop-2-en-1-ylidene] hydrazone (A7)

The mixture of 1H-indole-3-carbaldehyde hydrazone
(0.43g, 0.001 mol) and cinnamaldehyde (0.001 mol) in absolute alcohol (10 ml) was refluxed for 3 h. After TLC monitoring, reaction mixture was poured in crushed ice. The product formed was filtered, washed with cold water and recrystallized from ethanol.

Yield-65%, m.p-110°C, FT-IR 3186cm⁻¹ (NH stretch), 3050cm⁻¹ (Ar. C-H stretch), 2876cm⁻¹ (Aliphatic C-H stretch), 1566cm⁻¹ (C=C), 1617cm⁻¹ (C=N), Molecular formula- $C_{18}H_{15}N_3$.

General procedure for the synthesis of N-(1Hindol-3-ylmethyl) substituted aniline [B1-B16]

To the suspension of indole derivatives (0.005 mol) in DMF, formaldehyde (0.5 ml, 37%) and amines (0.005 mol) were added with vigorous stirring. The reaction solution was warmed for 20 min on a water bath and then allowed to cool. Then it was left at room temperature overnight. The solid precipitated was filtered and washed several times with ice cold water. The product was recrystallized using suitable solvent.

Synthesis of N-(1H-indol-3-ylmethyl) aniline [B1]

To the suspension of indole derivatives (0.005 mol) in DMF, formaldehyde (0.5 ml, 37%) and aniline (0.005 mol) were added with vigorous stirring. The reaction solution was warmed for 20 min on a water bath and then allowed to cool. Then it was left at room temperature overnight. The solid precipitated was filtered and washed several times with ice cold water. The product was recrystallized using ethanol.

 $\label{eq:2.1} \begin{array}{l} Yield\mathchar`^{1}(N\mathchar`^{1}(N\mathchar`^{1}(N\mathchar`^{1}(N\mathchar`^{1}(N\mathchar`^{1}(N\mathchar`^{1}(N\mathchar`^{1}(A\mathchar`)), A\mathchar`^{1}(A\mathchar`^{1}(A\mathchar`)), A\mathchar`^{1}(A\mathchar`^{1}(A\mathchar`)), A\mathchar`^{1}(A\mathchar`), A\mathchar`^{1}(A\mathchar`), A\mathchar`^{1}(A\mathchar`), A\mathchar`^{1}(A\mathchar`), A\mathchar`), A\mathchar`^{1}(A\mathchar`), A\mathchar`), A\mathchar`^{1}(A\mathchar`), A\mathchar`), A\mathchar`),$

Synthesis of N-(1H-indol-3-ylmethyl)-2-methyl aniline [B2]

To the suspension of indole derivatives (0.005 mol) in DMF, formaldehyde (0.5 ml, 37%) and o-Toluidine (0.005 mol) were added with vigorous stirring. The reaction solution was warmed for 20 min on a water bath and then allowed to cool. Then it was left at room temperature overnight. The solid precipitated was filtered and washed several times with ice cold water. The product was recrystallized using methanol.

Yield-81%, m.p-62-67°C, FT-IR 3398cm⁻¹ (N-H strech), 3031 cm^{-1} (Ar. C-H stretch), 2849 cm^{-1} (Aliphatic C-H stretch), 1516 cm^{-1} (C=C), 1394 cm^{-1} (C-N), Molecular formula- $C_{16}H_{16}N_2$.

Synthesis of N-(1H-indol-3-ylmethyl)-4-nitro aniline [B3]

To the suspension of indole derivatives (0.005 mol) in DMF,

formaldehyde (0.5 ml, 37%) and p-aniline (0.005 mol) were added with vigorous stirring. The reaction solution was warmed for 20 min on a water bath and then allowed to cool. Then it was left at room temperature overnight. The solid precipitated was filtered and washed several times with ice cold water. The product was recrystallized using methanol.

Yield-49%, m.p-120-125°C, FT-IR 3377cm⁻¹ (N-Hstrech), 3070cm⁻¹ (Ar. C-H stretch), 2903cm⁻¹ (Aliphatic C-H stretch), 1496cm⁻¹ (C=C), C-N strech (1311 cm⁻¹), 1597cm⁻¹ (C-NO₂), Molecular formula- $C_{15}H_{13}N_3O_2$.

Synthesis of N-(1H-indol-3-ylmethyl)-1,3benzothiazol-2-amine [B4]

To the suspension of indole derivatives (0.005 mol) in DMF, formaldehyde (0.5 ml, 37%) and 2-amino benzothiazole (0.005 mol) were added with vigorous stirring. The reaction solution was warmed for 20 min on a water bath and then allowed to cool. Then it was left at room temperature overnight. The solid precipitated was filtered and washed several times with ice cold water. The product was recrystallized using ethanol.

Yield-60%, m.p-150°C, FT-IR 3190cm⁻¹ (N-H stretch), 3096cm⁻¹ (Ar. C-H stretch), 2911cm⁻¹ (Aliphatic C-H stretch), 1534cm⁻¹ (C=C), 1341 cm⁻¹ (C-N strech), 1127 cm⁻¹ (C-S strech), 1607cm⁻¹ (C=N), Molecular formula- $C_{16}H_{13}N_3S$.

Synthesis of N-[(2-methyl-1H-indol-3-yl) methyl]-1, 3-thiazol-2-amine [B5]

To the suspension of methyl indole (0.005 mol) in DMF, formaldehyde (0.5 ml, 37%) and 2-amino thiazole (0.005 mol) were added with vigorous stirring. The reaction solution was warmed for 20 min on a water bath and then allowed to cool. Then it was left at room temperature overnight. The solid precipitated was filtered and washed several times with ice cold water. The product was recrystallized using methanol.

 $\begin{array}{l} \label{eq:2.1} Yield-69\%, \ m.p-170\,^{\circ}C, \ FT-IR \ 3394cm^{-1} \ (N-H \ strech), \\ 3356cm^{-1} \ (N-H \ strech), \ 3051cm^{-1} \ (Ar. \ C-H \ stretch), \ 2909cm^{-1} \\ (Aliphatic \ C-H \ strech), \ 1517cm^{-1} \ (C=C) \ , \ 1336 \ cm^{-1} \ (C-N \ strech), \ 1151 \ cm^{-1} \ (C-S \ strech), \ 1615 \ cm^{-1} \ (C=N), \ Molecular \ formula-C_{13}H_{13}N_3S. \end{array}$

Synthesis of 4-chloro-N-(1H-indol-3-ylmethyl)-1, 3-thiazol-2-amine [B6]

To the suspension of indole (0.005 mol) in DMF, formaldehyde (0.5 ml, 37%) and 4- chloro-2-amino thiazole (0.005 mol) were added with vigorous stirring. The reaction solution was warmed for 20 min on a water bath and then allowed to cool. Then it was left at room temperature

overnight. The solid precipitated was filtered and washed several times with ice cold water. The product was recrystallized using methanol.

Yield-62%, m.p-120°C, FT-IR 3394cm⁻¹ (N-H strech), 3356cm⁻¹ (N-H strech), 3051cm⁻¹ (Ar. C-H stretch), 2909cm⁻¹ (Aliphatic C-H stretch), 1517cm⁻¹ (C=C), 1336 cm⁻¹(C-N strech), 1151cm⁻¹(C-S strech), 1615cm⁻¹(C=N), Molecular formula-C₁₂H₁₀ClN₃S.

Synthesis of 4-chloro-N-[(2-methyl-1H-indol-3-yl) methyl]-1, 3-thiazol-2-amine [B7]

To the suspension of methyl indole (0.005 mol) in DMF, formaldehyde (0.5 ml, 37%) and 4- chloro-2-amino thiazole (0.005 mol) were added with vigorous stirring. The reaction solution was warmed for 20 min on a water bath and then allowed to cool. Then it was left at room temperature overnight. The solid precipitated was filtered and washed several times with ice cold water. The product was recrystallized using ethanol.

Yield-67%, m.p-122°C, FT-IR 3398cm⁻¹ (N-H strech), 3355cm⁻¹ (N-H strech), 3053cm⁻¹ (Ar. C-H stretch), 2902cm⁻¹ (Aliphatic C-H stretch), 1517cm⁻¹ (C=C), 1336 cm⁻¹ (C-N strech), 1164 cm⁻¹ (C-S strech), 1613cm⁻¹ (C=N), Mass 274 M⁺, Molecular formula- $C_{13}H_{12}ClN_3S$.

Synthesis of 4- methoxy-N-[(2-methyl-1H-indol-3yl) methyl] aniline [B8]

To the suspension of indole (0.005 mol) in DMF, formaldehyde (0.5ml, 37%) and p-anisidine (0.005 mol) were added with vigorous stirring. The reaction solution was warmed for 20 min on a water bath and then allowed to cool. Then it was left at room temperature overnight. The solid precipitated was filtered and washed several times with ice cold water. The product was recrystallized using methanol.

Yield-78%, m.p-105°C, FT-IR 3399cm⁻¹ (N-H strech), 3050cm⁻¹ (Ar. C-H stretch), 2909cm⁻¹ (Aliphatic C-H stretch), 1507cm⁻¹ (C=C), 1300cm⁻¹(C-N), 1233cm⁻¹ (C-O), Molecular formula- $C_{17}H_{18}N_2O$.

Synthesis of N-(1H-indol-3-ylmethyl)-4-phenyl-1, 3-thiazol-2-amine [B9]

To the suspension of indole (0.005 mol) in DMF, formaldehyde (0.5 ml, 37%) and 4-phenyl-2-amino thiazole (0.005 mol) were added with vigorous stirring. The reaction solution was warmed for 20 min on a water bath and then allowed to cool. Then it was left at room temperature overnight. The solid precipitated was filtered and washed several times with ice cold water. The product was recrystallized using methanol.

Yield-72%, m.p-100°C, FT-IR 3282cm⁻¹ (N-H strech), 3051 cm^{-1} (Ar. C-H stretch), 2900 cm^{-1} (Aliphatic C-H stretch), 1457 cm^{-1} (C=C), 1336 cm^{-1} (C-N strech), 1152 cm^{-1} (C-S strech), 1613 cm^{-1} (C=N), Molecular formula- $C_{18}H_{15}N_3O$.

Synthesis of N-[(2-methyl-1H-indol-3-yl)methyl]-4-phenyl-1, 3-thiazol-2-amine [B10]

To the suspension of methyl indole (0.005 mol) in DMF, formaldehyde (0.5 ml, 37%) and 4-phenyl-2-amino thiazole (0.005 mol) were added with vigorous stirring. The reaction solution was warmed for 20 min on a water bath and then allowed to cool. Then it was left at room temperature overnight. The solid precipitated was filtered and washed several times with ice cold water. The product was recrystallized using methanol.

Yield-56%, m.p-100°C, FT-IR 3397cm⁻¹ (N-H strech), 3357cm⁻¹ (N-H strech), 3052cm⁻¹ (Ar. C-H stretch), 2906cm⁻¹ (Aliphatic C-H stretch), 1525cm⁻¹ (C=C), 1336 cm⁻¹ (C-N strech , 1152 cm⁻¹ (C-S strech), 1611cm⁻¹ (C=N), Molecular formula- $C_{19}H_{17}N_3S$.

Synthesis of N-(1H-indol-3-ylmethyl)-3, 3'dimethoxybiphenyl-4,4'-diamine [B11]

To the suspension of indole (0.005 mol) in DMF, formaldehyde (0.5 ml, 37%) and o-dianisidine (0.005 mol) were added with vigorous stirring. The reaction solution was warmed for 20 min on a water bath and then allowed to cool. Then it was left at room temperature overnight. The solid precipitated was filtered and washed several times with ice cold water. The product was recrystallized using ethanol.

Yield-77%, m.p-88°C, FT-IR 3401cm⁻¹ (N-H strech), 3049cm⁻¹ (Ar. C-H stretch), 2944cm⁻¹ (Aliphatic C-H stretch), 1577cm⁻¹ (C=C), 1609cm⁻¹ (C=N), 1237 cm⁻¹ (C-O), 1332 cm⁻¹ (C-N stretch), Molecular formula- $C_{22}H_{23}N_3O_2$.

Synthesis of 3, 3'-dimethoxy- N-[(2-methyl-1Hindol-3-yl) methyl] biphenyl-4, 4'-diamine [B12]

To the suspension of methyl indole (0.005 mol) in DMF, formaldehyde (0.5 ml, 37%) and o-dianisidine (0.005 mol) were added with vigorous stirring. The reaction solution was warmed for 20 min on a water bath and then allowed to cool. Then it was left at room temperature overnight. The solid precipitated was filtered and washed several times with ice cold water. The product was recrystallized using ethanol.

Synthesis of N-(1H-indol-3-ylmethyl)-1, 5-dihydro-4H-1, 2, 4-triazol-4-amine [B13]

To the suspension of indole (0.005 mol) in DMF, formaldehyde (0.5 ml, 37%) and 4-amino-1,2,3-triazole (0.005 mol) were added with vigorous stirring. The reaction solution was warmed for 20 min on a water bath and then allowed to cool. Then it was left at room temperature overnight. The solid precipitated was filtered and washed several times with ice cold water. The product was recrystallized using ethanol.

Yield-69, m.p-87°C, FT-IR 3395cm⁻¹ (N-H strech), 3048cm⁻¹ (Ar. C-H stretch), 2837cm⁻¹ (Aliphatic C-H stretch), 1500cm⁻¹ (C=C), 1610cm⁻¹ (C=N), 1338 cm⁻¹ (C-N stretch), Molecular formula- $C_{11}H_{13}N_5$.

Synthesis of N-[(2-methyl-1H-indol-3-yl) methyl]-4H-1, 2, 4-triazol-4-amine [B14]

To the suspension of methyl indole (0.005 mol) in DMF, formaldehyde (0.5 ml, 37%) and 4-amino-1,2,3-triazole (0.005 mol) were added with vigorous stirring. The reaction solution was warmed for 20 min on a water bath and then allowed to cool. Then it was left at room temperature overnight. The solid precipitated was filtered and washed several times with ice cold water. The product was recrystallized using ethanol.

Yield-84, m.p-90°C, FT-IR 3387cm⁻¹ (N-H strech), 3049cm⁻¹ (Ar. C-H stretch), 2907cm⁻¹ (Aliphatic C-H stretch), 1545cm⁻¹ (C=C), 1612cm⁻¹ (C=N), 1336 cm⁻¹ (C-N stretch), ¹HNMR (CD₃OD, TMS): 7.8 (s, NH of indole); 7.5 (s, NH); 7-7.25 (m, aromatic); 6.2 (s, 1H CH2), Molecular formula- $C_{12}H_{13}N_5$.

Synthesis of 4-amino-N-(7, 7 a-dihydro-1H-indol-3-ylmethyl)benzenesulfonamide [B15]

To the suspension of indole (0.005 mol) in DMF, formaldehyde (0.5 ml, 37%) and sulphanilamide (0.005 mol) were added with vigorous stirring. The reaction solution was warmed for 20 min on a water bath and then allowed to cool. Then it was left at room temperature overnight. The solid precipitated was filtered and washed several times with ice cold water. The product was recrystallized using ethanol.

Yield-75, m.p-75°C, FT-IR 3394cm⁻¹ (N-H strech), 2929cm⁻¹ (Aliphatic C-H stretch), 1597cm⁻¹ (C=C), 1318cm⁻¹ (C-N), Molecular formula- $C_{15}H_{15}N_3O_2S$.

Synthesis of 4-amino-N-[(2-methyl-7, 7a-dihydro-1H-indol-3-yl) methyl] benzenesulfonamide [B16]

To the suspension of methyl indole (0.005 mol) in DMF, formaldehyde (0.5 ml, 37%) and sulphanilamide (0.005 mol)

were added with vigorous stirring. The reaction solution was warmed for 20 min on a water bath and then allowed to cool. Then it was left at room temperature overnight. The solid precipitated was filtered and washed several times with ice cold water. The product was recrystallized using methanol.

Yield-70, m.p-115-120°C, FT-IR 3390cm⁻¹ (N-H strech), 2941cm⁻¹ (Aliphatic C-H stretch), 1597cm⁻¹ (C=C), 1314cm⁻¹ (C-N), Molecular formula- $C_{16}H_{17}N_3O_2S$.

Biological screening

Antimicrobial activity^[43-44]:

The antibacterial activity of synthesized Schiff's bases was tested against B. subtilis and E. coli using Muller-Hinton nutrient agar medium. The antifungal activity of synthesized Schiff's bases was tested against *Penicillium notatum* and *Aspergillus niger* using Sabouraud's nutrient agar medium.

Cup plate method:

The medium was prepared and sterilized [autoclaved at 120°C for 30 min] then the medium (40-50°C) was poured into Petri plates and allowed to solidify. Later suspension of the microorganism was inoculated onto the medium using a sterile cotton swab. 6 mm diameter stainless steel disc is used to remove the agar from the medium. Three cups were made in each plate at equal distances. One cup was filled with 0.1 ml of standard drug, other with 0.1 ml of DMSO; others were filled with 0.1 ml of test compounds. The plates were preincubated for 1 h at room temperature and incubated at 37°C for 24 and 48 h for antibacterial and antifungal activity respectively. Amoxycillin, Fluconazole were used as standard for antibacterial and antifungal activity respectively. The observed zone of inhibition for antibacterial and antifungal activity is presented in Table land 2 respectively. The standard and tested compounds were suspended in DMSO.

Pharmacological screening

Anti-Inflammatory activity study^[45-46]

Carrageenan-induced paw edema model

Albino rats of Wistar strain of either sex were used in experiments. The animals were kept in the groups (control, treated, standard) under constant temperature (25±1°C) and 12 h light/dark cycle. They had free access to standard mouse diet and tap water except during the experiment. On the day of the experiment, animals were transferred to individual cages randomly and allowed to acclimatize for 30 min before drug administration. Diclofenac sodium was used as standard drugs. Newly synthesized compounds were dissolved in propylene glycol. Anti-inflammatory activity

Percent

Paw oedema inhibition test was performed on albino rats. Thirty min later, 0.2ml of 1% Carrageenan suspension in 0.9% NaCl solution was injected subcutaneously into the plantar aponeurosis of the hind paw, and the paw volume was measured by the aid of plethysmometer and then measured at 30, 60, 90, 120 min later. The mean increase of

Table1: Results of Antibacterial stud					
S1.	Comp	Conc	Zone of Inhibi	tion (mm)	
No.	Code	μg/ml	B. subtilis	E. coli	
1	Al	800	11	8	
		1000	12	8	
		1200	10	7	
2	A2	800	6	2	
		1000	6	7	
		1200	6	8	
3	A3	800	7	8	
		1000	9	8	
		1200	8	5	
4	A4	800	7	2	
		1000	7	2	
		1200	6	2	
5	STANDARD	800	13	8	
		1000	12	6	
		1200	12	7	

NOTE: NI= no inhibition, Control= DMSO, 1-4 mm= poor activity, 4-7mm= moderate activity, above 7mm=good activity.

Table 2: Results of Antifungal stud					
Sl. No.	Comp Code	Conc µg/ml	Zone of Inhi <i>Penicillium</i>	bition (mm) Aspergillus	
1	Al	800	12	9	
		1000	13	10	
		1200	12	9	
-2	A2	800	7	2	
		1000	8	2	
		1200	7	2	
3	A3	800	7	2	
		1000	8	2	
		1200	7	2	
4	A4	800	2	2	
		1000	6	2	
		1200	6	2	
5	STANDARD	800	12	11	
		1000	11	10	
		1200	11	11	
NOTE: NI= no inhibition, Control= DMSO, 1-4 mm= poor activity, 4-7mm= moderate activity, above 7mm=good activity.					

paw volume at each time interval was compared with that of control group at the same time intervals and percent inhibition values were calculated by the formula given below:

$$(V_{t-}V_o)$$
 control - $(V_{t-}V_o)$ treated
age of inhibition =----- **X**100

 $(V_{t-}V_{o})$ control

Where Vt and Vc are mean increase of paw volume of test and control groups, respectively.

The mean increase in paw volume and percentage inhibition of paw edema is mentioned in table 3 and 4 respectively.

Analgesic activity^[43]

Acetic-acid induced writhings in mice

Acetic acid writhing test was performed on Albino mices of Swiss strain. Test compounds were given to the animals at a dose of 50 mg/kg, 30 min later the animals were injected intraperitoneally with 0.25 ml/mouse of 0.5% acetic acid. The mean number of writhes for each experimental groups and percentage decrease compared with the control group were calculated after 60 min. The analgesic activity readings are mentioned in table 5.

RESULTS

Indole-3-carbaldehyde was treated with hydrazine hydrate to form 1H-indole-3-carbaldehyde hydrazone, which upon treatment with substituted aldehydes give 1H -indolo-3carbaldehyde [substituted methylene] hydrazine. Substituted indole derivatives on treatment with formaldehyde and substituted amines in presence of DMF to form N-(1H-indol-3-ylmethyl) substituted aniline. The reaction was well monitored through thin layer chromatography technique. The synthesized compounds were dried and recrystallized from ethanol. The antibacterial, antifungal activity of Schiff's bases and analgesic, anti-inflammatory activities of Mannich bases was carried out.

DISCUSSION

Schiff's bases were synthesized from Indole-3-carbaldehyde through 1H-indole-3-carbaldehyde hydrazone (A). The synthesized compounds were confirmed by IR, ¹H NMR and Mass spectral means. The compounds were evaluated for antimicrobial activities. The derivative A1 at 800 μ g/ml, 1000 μ g/ml showed good antibacterial against *B. subtilis* and at 800 μ g/ml, 1000 μ g/ml and 1200 μ g/ml showed good antifungal activity.

D. Giles et al Synthesis and Pharmacological evaluation of Schiff's and Mannich bases of Indole Derivatives

Table 3: Results of Anti-Inflammator activit							
	Mean increase in paw volume (mean ± SEM)						
Treatment _	Time in minutes						
	30	60	90	120			
Control	0.052 ± 0.00068	0.0717 ± 0.0055	$0.097 {\pm} 0.00047$	0.124 ± 0.0002			
Std.	$0.022 \pm 0.00054^{**}$	$0.026 \pm 0.00033^{**}$	$0.023 \pm 0.0002^{**}$	$0.02 \pm 0.0003^{**}$			
Group-I (B1)	$0.049 \pm 0.00048^{**}$	$0.066 {\pm} 0.0006^{**}$	$0.09 \pm 0.00047^{**}$	0.12 ± 0.004^{ns}			
Group-II (B2)	$0.0542 {\pm} 0.00065^{ns}$	$0.075 {\pm} 0.0011^{*}$	$0.098 {\pm} 0.0005^{ m ns}$	0.12 ± 0.006^{ns}			
Group-III (B3)	$0.0413 \pm 0.00067^{**}$	$0.0555 {\pm} 0.00062^{**}$	$0.079 {\pm} 0.0008^{**}$	0.11 ± 0.005^{ns}			
Group-IV (B4)	0.0517 ± 0.00013^{ns}	0.0703 ± 0.0001^{ns}	0.095 ± 0.0001^{ns}	0.15 ± 0.0175^{ns}			
$Group\text{-}V\left(B5\right)$	$0.0547 {\pm} 0.00084^{ns}$	$0.0752 {\pm} 0.00075^{**}$	0.096 ± 0.0006^{ns}	0.121 ± 0.0002^{ns}			
Group-VI (B6)	$0.0498 {\pm} 0.0006^{ns}$	0.0721 ± 0.00047^{ns}	$0.095 {\pm} 0.0007^{ m ns}$	0.119 ± 0.0005^{ns}			
Group- VII (B7)	$0.0502 {\pm} 0.00079^{ m ns}$	$0.068 {\pm} 0.00068^{**}$	$0.093 \pm 0.0008^{**}$	0.12 ± 0.0005^{ns}			
Group- VIII (B8)	0.05 ± 0.00036^{ns}	$0.0683 {\pm} 0.00049^{*}$	$0.093 \pm 0.0006^{**}$	0.13 ± 0.015^{ns}			
Group-IX (B9)	$0.0467 \pm 0.00084^{**}$	$0.062 \pm 0.00073^{**}$	$0.085 \pm 0.0002^{**}$	0.12 ± 0.0006^{ns}			
Group-X (B10)	0.0517 ± 0.00067^{ns}	$0.0728 {\pm} 0.00065^{ m ns}$	$0.095 {\pm} 0.0006^{ns}$	0.120 ± 0.0007^{ns}			

Statistical analysis was done by ANOVA followed by Dunnet s test. All the values are expressed as mean ± SEM *P<0.05, **P<0.01. When compared to control ns: non-significant

Table 4: Anti-inflammator activit (Percentage inhibition of paw volume)						
Treatment	(Percentage Inhibition of Paw edema)					
]	lime in m	inutes			
	30	60	90	120		
Std.	50%	65%	76%	80%		
Group-I (B1)	9.6%	6.8%	4.12%	2.4%		
Group-II (B2)	Ns	Ns	Ns	Ns		
Group-III (B3)	23%	26	30	11.5		
$Group\text{-}IV\left(B4\right)$	7.6%	6.8%	4.1%	2.4%		
Group-V $(B5)$	Ns	Ns	Ns	Ns		
Group-VI (B6)	13%	16.4%	17.5%	0.8%		
Group-VII (B7)	7.6%	6.7%	4.1%	2.4%		
Group- VIII (B8)	5.7%	8.2%	3.9%	3.3%		
Group-IX (B9)	Ns	Ns	Ns	Ns		
Group-X (B10)	Ns	Ns	Ns	Ns		
Ns: Non-significant						





Table 5: Results of Analgesic activit				
Treatment	Number of Writhings			
Control	51 ± 0.54			
Diclofenac Sodium	25±0.614**			
Group-I (B-01)	$44.66 \pm 0.42^{*}$			
Group-II (B-02)	$47.66 {\pm} 0.33^{*}$			
Group-III (B-03)	$34.66 \pm 0.557^*$			
Group-IV (B-04)	$47.66 {\pm} 0.494^{*}$			
$Group\text{-}V\left(B\text{-}05\right)$	$43.16 \pm 0.307^*$			
Group-VI (B-06)	$36.66 {\pm} 0.33^{*}$			
Group-VII (B-07)	34.16±0.749*			
Group-VIII (B-08)	$43.83 \pm 0.477^*$			
Group-IX (B-09)	$48 {\pm} 0.365^{*}$			
Group-X $(B-10)$	$43.16 \pm 0.477^{*}$			
Statistical analysis was done by ANOVA followed by Dunnet s test. All the values are expressed as mean + SEM * $P_{<0}05$ ** $P_{<0}01$				

When compared to control

Substituted indole derivatives were treated with formaldehyde and substituted amines in presence of DMF to form N-(1H-indol-3-ylmethyl) substituted aniline (B1-B16). The synthesized compounds were dried and recrystallized from suitable solvent. Confirmations of the synthesized compounds were done by IR, ¹H NMR and Mass spectral analysis. The synthesized compounds were evaluated for analgesic and anti-inflammatory activities. Derivatives B3, B6 have shown significant antiinflammatory and analgesic activity.

CONCLUSION

It was observed that the new synthesized compound A1, A2, A3 possessing electron withdrawing group like nitro exhibited good antibacterial at 1000 μ g/ml and moderate activity1200 μ g/ml, 1000 μ g/ml respectively and antifungal activity at 1000 μ g/ml for compound A1 than other compounds. It was observed that the newly synthesized Mannich bases like B3, B6, and B7 possessing electron withdrawing groups like nitro, chloro, exhibits better anti-inflammatory and analgesic activity. It clearly indicates that the effect of pharmacophore role in the pharmacological activity.

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Antiulcer activity of Ethanolic extract of Gossypium Harbaceum flowers

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The present study was undertaken to determine the antiulcer potential of ethanolic extract of flowers of *Gossypium* harbaceum L. (EEGH) using ethanol induced acute gastric ulcers in rats. EEGH were tested orally at the doses of 250 mg/kg and 500 mg/kg on gastric ulcerations experimentally induced by ethanol model. The cytoprotection hypotheses were evaluated for antiulcer effect. The ethanolic extract at 250 and 500 mg/kg has significantly inhibited ulcer formation. There was a significant (p<0.001) dose- dependent decrease in the ulcerative lesion index produced by this model as compared to the standard drug lansoprazole (8 mg/kg, b.w. orally). The antiulcer effect of EEGH was indicted by the reduction in the ulcer index and significant prevention of gastric mucosal damage induced by ethanol. This was also revealed by examining the gastric tissue histopathologically. The EEGH at 500 mg/kg has shown antiulcer activity almost equivalent to that of lansoprazole. The antiulcer property may be related to the tannins and flavonoids present in the extract. This result clearly indicates that the ethanolic extract of flowers of *Gossypium herbaceum* L. increases healing of gastric ulcer and possess potential antiulcer activity than compare to control and standard group.

Keywords: Gossypium herbaceum flower; Antiulcer activity; Ulcer index; Ethanol induced ulcer; Histopathology.

INTRODUCTION

Peptic ulcer is one of the major gastro-intestinal disorders, which occur due to an imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors¹. Consequently, reduction of gastric acid production as well as re-enforcement of gastric mucosal production has been the major approaches for therapy of peptic ulcer disease. As a result, more and more drugs, both herbal and synthetic are coming up offering newer and better options for treatment of peptic ulcer. The type of drugs varies from being proton-pump inhibitor to H₂ antagonist or a cytoprotective agent. At the same time, each of these drugs confers simpler to several side effects like arrhythmias, impotence, gynaecomastia, enterochromaffinlike cell (ECL), hyperplasia and haemopoeitic changes². The prevalence rate is approx 1 in 54 or 1.84% or 5 million people in USA³.

Medicinal plants have been used to treat variety of gastrointestinal disorders such as gastric ulcer, gastric carcinoma, constipation, diarrhoea. Recently, widespread effort has been launched to identify novel anti-ulcer drugs from natural resources. The study assumes significance in the context that prolonged use of synthetic anti-ulcer drugs leads to adverse drug reactions and a search for new anti-

RGUHS Journal of Pharmaceutical Sciences Received: 31/01/2011, Modified on: 23/02/2011, Accepted on: 01/03/2011 ulcer agents that retain therapeutic efficacy and are devoid of adverse drug reaction are warranted. The natural approach to heal ulcers is to identify and reduce all factors that can contribute to their development. These factors are low-fiber diet, cigarette smoking, stress, and drugs such as aspirin and other non-steroidal anti-inflammatory drugs⁴. Inflamed break in the lining of the stomach or the duodenum caused due to either increased acid production or damage to the mucus lining of the stomach leads to formation of peptic ulcer, a term that includes both gastric as well as duodenal ulcer. Secondary causes of ulcer may be due to the use of conventional non-steroidal anti inflammatory drugs (NSAID's) and current gastric and duodenal ulcers are caused by "Helicobacter pylori". The common clinical features of peptic ulcer are hyper acid secretions and ulcer formation in stomach and duodenal part of intestine⁶. Reduction of gastric acid production as well as re-enforcement of gastric mucosal production has been the major approaches for therapy of peptic ulcer disease. As a result, more and more drugs, both herbal and synthetic are coming up offering newer and better options for treatment of peptic ulcer. The type of drugs varies from being proton pump inhibitors to H₂-antagonists or cytoprotective agent.

Many such herbs, shrubs and plants are known to protect the organs from the environmental, chemical and occupational challenges. *Gossypium herbaceum* Linn. is one such green leaf plant. The ethnobotanical studies and folklore's claiming

revealed that the *Gossypium herbaceum* is used in various therapeutic properties such as diuretic, haematinic, laxative, expectorant in action and it is also used in Ayurvedic formulations. *Gossypium herbaceum* contains flavonoids, tannins, steroids, terpine aldehyde, alkaloids, carbohydrate and protein⁷. The objective of the present study was to investigate the anti-ulcer property of the plant extract.

MATERIALS AND METHODS

The fresh flowers of *Gossypium herbaceum* Linn were collected from Kajala Dist. Osmanabad (Maharashtra). It was authenticated by Mr. P. G. Diwakar, Joint Director, Botanical Survey of India, Western circle-7, Koregaon Road, Pune-1 with an voucher No. KAHG 01, dated 06/08/2009.

Extraction⁸

The fresh flowers were shade-dried and made into a coarse powder which was passed through a 40-mesh sieve to get a uniform particle size and then used for extraction. A weighed quantity (125 g) of the powder was then subjected to continuous hot extraction in Soxhlet apparatus with increasing order of polarity i.e. petroleum ether (60-80° C), chloroform (59.5-61.5° C), ethanol 64.5-65.5° C) and distilled water. The extracts obtained were concentrated under reduced pressure to give a yield of 3.8 %. Preliminary phytochemical analysis was carried out to identify presence of phytoconstituents in the crude extract.

Preliminary phytochemical analysis⁹

The ethanolic extract of *Gossypium herbaceum* Linn was then subjected to preliminary phytochemical analysis to assess the presence of various Phytoconstituents. It revealed that the presence of tannins and phenolic compounds along with the presence of flavonoids, saponins, glycosides, steroids, alkaloids and Carbohydrates. See Table 1.

Animals

Albino mice of either sex weighing between 20-25 g and albino rats of Wistar strain of either sex weighing between 150-200 g procured from Mahavir Enterprises, Hyderabad were used for the present investigation. The rats were fed on a standard pellet diet (Hindustan Lever Ltd., Bangalore, India) and water *ad libitum* and maintained at 25°C with 12 hr light / dark cycle. After laboratory acclimation for 7 days, the rats were starved for 48 hr. Prior approval by institutional ethics committee (reg.no: 346/CPCSEA) was obtained for conduction of experiments. The animals were deprived of food for 24hrs before experimentation, but had free access to drinking water. The study was conducted in the

Table 1: Preliminary Phytochemical screening					
S1.	Types of phytochemical	Ethanolic			
No.	constituents	extract			
1	Carbohydrate	+++			
2	Proteins	-			
3	Flavonoids	++			
4	Steroids	++			
5	Tannins and phenolic compounds	+++			
6	Saponins glycosides	++			
7	Cardiac glycosides	-			
8	Starch	-			
9	Alkaloids	++			
-Absent, + Indicates presence, ++ More clarity, +++ Better response					

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Drugs and chemicals

Lansoprazole was obtained from Lee Pharmaceuticals, Hyderabad. Ethanol was obtained from Changshu Yangyuan Chem. China. All other chemicals used in this study were obtained commercially and were of analytical grade.

Acute toxicity studies¹⁰

Acute toxicity studies for ethanolic extracts of *Gossypium herbaceum* L. belonging to the family Malvaceae were conducted as per OECD guidelines 420 using albino Swiss mice. Each animal was administered ethanolic extract by oral route. The animals were observed for any changes continuously for the first 2 hours and up to 14days for mortality. There were no mortality and noticeable behavioral changes in all the groups tested. The extracts were found to be safe up to 2000 mg/kg body weight.

Selection of dose of the extract

An attempt was made to identify LD_{50} of ethanolic extract of *Gossypium herbaceum* flowers. Since no mortality was observed at 2000 mg/kg, it was thought that 2000 mg/kg was the cut off dose. Therefore, 1/8 and 1/4 dose i.e. 250 mg/kg and 500 mg/kg body weight were selected for all further in vivo studies.

Antiulcer activity

Ethanol induced ulcers¹¹

Albino wistar rats of either sex weighing between 150-200 g were divided into four groups of six animals each. Group-I was administered absolute ethanol (1ml/200 g, per oral) and served as control. Group-II served as standard and received

Lansoprazole (8 mg/kg p.o).). Group-III and IV were given orally ethanolic extract (250 and 500 mg/kg) of *Gossypium herbaceum* flowers respectively. They were fasted overnight before the test with free access to water.

One hour after the administration of extract and standard drug, absolute ethanol at dose of 1 ml/200 g was administered to all the animals. The animals were sacrificed 1h after administration of ethanol and the stomach was removed and opened along the greater curvature. Lesions were examined with the help of hand lens (10X) and sample was sending to further histopathological study. Scoring was done as, 0 = normal stomach, 0.5 = red coloration, 1.0 = spot ulcers, 1.5 = hemorrhagic streaks, 2.0 = ulcer > 3 but < 5 and 3.0 = ulcer > 5.

Mean ulcer score for each animal is expressed as ulcer index. The percentage protection was calculated using the following formula;

Percentage protection = $100 - Ut / Uc \times 100$

Where, Ut = ulcer index of treated group.

Uc = ulcer index of control group

Histopathological evaluation¹²

The rats were sacrificed by deep ether anesthesia. The stomach was isolated and preserved in 10% formaldehyde solution for fixation. Then the organ was washed in running tap water for 6-7 hours to remove excess of formaldehyde. Then the organ in capsule was kept in ascending grades of alcohol (70%, 80%, 90% and 95% for 12-15 hours and in absolute alcohol for 6 hours) to remove water from tissue. The clearing of tissue was done by keeping the capsule in Xylene solution for 10-15 minutes. Stomach was then embedded in paraffin wax by boiling for 2 hours and was enclosed in paraffin block. Finally the sections of 4-6 microns were cut on microtome machine. Slides were stained by heamatoxylin eosin staining method and these were examined under the microscope for histopathological changes such as congestion, hemorrhage, necrosis, inflammation, infiltration, erosion and ulcer and photographs were taken. See Figure 3.

Statistical analysis

Results were expressed as mean±SEM, (n=6). Statistical analysis were performed with one way analysis of variance (ANOVA) followed by Dunnett's 't' test P value less than <0.05 was considered to be statistically significant. *P<0.05, **P<0.01 and ***P<0.001, when compared with control and toxicant group as applicable.

RESULTS

The preliminary phytochemical investigation has revealed that the ethanolic extract of flowers of *Gossypium herbaceum* L. said to contain tannins, flavonoids, steroids, carbohydrates, vitamins and alkaloids.

Selection of dose of the extract

 Ld_{50} was done as per OECD guidelines for fixing the dose for biological evaluation. In LD_{50} studies, it was found that the animals were safe up to a maximum dose of 2000 mg/kg body weight. There were no changes in normal behavior pattern and no signs and symptoms of toxicity and mortality were observed. The biological evaluation was carried out at doses (1/8th and 1/4th of LD_{50} cut off values) of 250 and 500 mg/kg body weight.

Rats when treated with ethanolic extract of *Gossypium* herbaceum L. with a dose of 250 mg/kg and 500 mg/kg, produced a dose dependent protection in the ethanol induced ulceration model as compared to control group. However the protection was statistically significant (P<0.05, P<0.01, P<0.001), to reduce the severity of ulcer and caused a significant reduction of ulcer index in this model. Lansoprazole produced significant gastric ulcer protection as compared to control group.

The reduction of ulcer index at a dose 8 mg/kg of Lansoprazole, 250 mg/kg and 500 mg/kg of EEGH, were 1.66, 4.83 and 2.08 respectively.

The percentage protection at a dose 8mg/kg of Lansoprazole, 250 mg/kg and 500 mg/kg of EEGH were 76.28%, 31.00% and 70.28% respectively.

The ulcer protective action at 500 mg/kg dose of EEGH was found to be closer to the reference standard drug, Lansoprazole. The results are tabulated in Table 2. The photographs of open stomach are affixed in Figure 1.

Histopathological studies of ethanolic extract of *Gossypium herbaceum* flowers on absolute ethanol induced ulcer in rat have shown the following results and depicted in Figure 2. The mucosa in control group (absolute ethanol 1ml/200 g) showed redness, infiltration, congestion, hemorrhagic sticks, inflammation, necrosis and dilation of blood vessel, while in Lansoprazole (8 mg/kg p. o.) treated group, the mucosa showed the mild redness, no inflammation, and dilation of blood vessels. Group III at 250 mg/kg p. o. of EEGH treated group, the mucosa showed the mild redness, mild inflammation and no congestion, where in group IV, 500 mg/kg of EEGH treated group, mucosa showed the mild

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Table 2: Effect of ethanolic extract of <i>Gossypium herbaceum</i> flowers on ulcer index and their % protection in ethanol induced ulcer in rats.						
Group no	Treatment	Dose	Ulcer index	% protection		
1	Control (absolute ethanol)	1 ml/200 g, p.o.	7.00±0.632			
2	Lansoprazole	8 mg/kg, p.o	1.66±0.459***	76.28		
3	EEGH	250 mg/kg, p.o	4.83±0.749*	31.00		
4	EEGH	500 mg/kg, p.o	2.08±0.436***	70.28		
Values are the mean S.E.M. of 6 rats / treatment, Significant *P < 0.05, **P < 0.01 and ***P < 0.001 compared with Control.						

Fig.1: Effect of ethanolic extract of Gossypium herbaceum flowers in ethanol induced ulcer in rats.

Control Ethanol



250 mg/kg EEGH





500 mg/kg EEGH





Fig. 2: Histological study of ethanolic extract of Gossypium herbaceum (EEGH) flowers on Absolute ethanol induced ulcer in rats.

Control Ethanol



250 mg/kg EEGH



dilation of blood vessels mild redness, inflammation, congestion, hemorrhagic sticks and necrosis.

DISCUSSION

The anti-ulcer activity of the plant of Gossypium herbaceum Linn was evaluated by ethanol induced ulceration. The model represents some of the most common causes of gastric ulcer in humans. Many factors and mechanisms are implicated in the ulcerogenesis and gastric mucosal damage induced by ethanol induced ulceration model employed in the present study involving, free radical production. Prostaglandins (PG) offer protection to stomach through both increases in mucosal resistance as well as decrease in aggressive factors, mainly acid and pepsin¹³. Ethanol induced gastric ulcers have been widely used for the evaluation of gastro protective activity. Ethanol is metabolized in the body and releases superoxide anion and hydroperoxy free radicals. It is associated with significant production of oxygen free radicals leading to increased lipid peroxidation, which causes damage to cell and cell





500 mg/kg EEGH



membrane¹⁴. The incidence of ethanol induced ulcers is predominant in the glandular part of stomach. It was reported to stimulate the formation of leukotriene C4 (LTC4), mast cell secretory products and reactive oxygen species resulting in the damage of rat gastric mucosa¹⁵. It has been found that oxygen derived free radicals are implicated in the mechanism of acute and chronic ulceration in the gastric mucosa and scavenging these free radicals can play an appreciable role in healing these ulcer¹⁶. The ethanolic extract of the Gossypium herbaceum Linn with a dose 250 mg/kg and 500 mg/kg has significantly protected the gastric mucosa against ethanol challenge as shown by reduced values of lesion index as compared to control group suggesting its potent cytoprotective effect. The preliminary phytochemical studies revealed the presence of tannin, phenolic compounds and flavonoids in ethanolic extract of Gossypium herbaceum Linn. Various tannin, phenolic compounds and flavonoids have been reported for its antiulcerogenic activity with good level of gastric protection. So

the possible antiulcer property of *Gossypium herbaceum* Linn may be due to its tannin, phenolic compounds and flavonoid content. In this study we observed that *Gossypium herbaceum* Linn provides significant anti-ulcer activity against gastric ulcers in rats.

CONCLUSION

The phytochemical studies of *Gossypium herbaceum* L. flowers show the presence of tannins, flavonoids, steroids, carbohydrates, vitamins and alkaloids. On the basis of the present results and available reports, it can be concluded that the anti-ulcer activity elucidated by *Gossypium herbaceum* L. could be mainly due to the modulation of defensive factors through an improvement of gastric cytoprotection. At 500 mg/kg ethanolic extract has reduced ulcer index more significantly than 250 mg/kg when compared with the control and standard group.

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Preparation and *In Vitro* Evaluation of Acyclovir Loaded Eudragit[®] RLPO Nanoparticles as Sustained Release Carriers

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Acyclovir is an antiviral drug, used for treatment of herpes simplex virus infections with an oral bioavailability of only 10 to 20 % (limiting absorption in GIT to duodenum and jejunum), half life about 3h, soluble only at acidic pH (pKa 2.27). Polymeric nanodrug delivery systems of acyclovir have been designed and optimized using 3² full factorial design. Eudragit RLPO was used as polymer and Pluronic F68 as stabilizer. From the preliminary trials, the constraints for independent variables X₁ (drug: polymer ratio), X₂ (amount of stabilizer i.e. Pluronic F68) have been fixed. The dependent variables that were selected for study were, particle size (Y₁) and % drug entrapment (Y₂). The derived polynomial equations were verified by check point formulation. The application of factorial design gave a statistically systematic approach for the formulation and optimization of nanoparticles with desired particle size, % drug release and high entrapment efficiency. Drug:polymer ratio and concentration of stabilizer were found to influence the particle size and entrapment efficiency of acyclovir loaded Eudragit RLPO nanoparticles. The release was found to follow non-Fickian diffusion mechanism with first order drug release for all batches. These preliminary results indicate that acyclovir loaded Eudragit RLPO nanoparticles could be effective in sustaining drug release for a prolonged period.

Keywords: Acyclovir, Eudragit[®] RLPO, nanoparticles, 3² factorial design, response surface plots.

INTRODUCTION

Herpes simplex virus (HSV) is a member of family of herpes viridae, a DNA virus. There are two types of Herpes Simplex Viruses (HSV). viz HSV type 1 and type 2. HSV type 1 is the herpes virus that is usually responsible for cold sores of the mouth, the so called, fever blisters. HSV type 2 is the one that most commonly causes genital herpes^[1]. The infection causes painful sores on the genitals in both men and women. In addition, Herpes sores provide a way for HIV to get past the body's immune defenses and make it easier to get HIV infection. A recent study found that people with HSV had three times the risk of becoming infected with HIV as compared to people without HSV^[2]. Currently the treatments available for herpes simplex are conventional tablets and topical gel for application on outbreaks. The drugs that are commonly used for herpes simplex are acyclovir, valaclovir and famciclovir.

Acyclovir, the first agent to be licensed for the treatment of herpes simplex virus infections, is the most widely used drug for infections such as cutaneous herpes, genital herpes, chicken pox, varicella zoster infections. Acyclovir is currently marketed as capsules (200 mg), tablets (200 mg,

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400 mg and 800 mg) and topical ointment^[1]. Oral acyclovir is mostly used as 200 mg tablets, five times a day. In addition, long term administration of acyclovir (6 month or longer) is required in immunocompromised patient with relapsing herpes simplex infection^[2]. The presently available conventional therapy is associated with a number of drawbacks such as highly variable absorption and low bioavailability (10–20%) after oral administration^[3]. Furthermore, with increase in dose, there is decrease in bioavailability. Moreover, because the mean plasma half life of the drug is 2.5 h, five times a day administration is required. In order to make oral therapy of acyclovir more patient compliant there is a need of using different approaches like matrix tablets, nanoparticles^[4] and polymeric films^[5].

The main problem with the therapeutic effectiveness of acyclovir is its absorption which is highly variable and dose dependent, thus reducing the bioavailability to $10-20\%^{[6]}$. Acyclovir is soluble in acidic pH and is predominantly absorbed from upper gastro-intestinal tract (GIT)^[7]. There are indications of its active absorption from the duodenum and jejunum regions of GIT^[8]. The inherent shortcomings of conventional drug delivery and the potential of nanoparticles as drug delivery systems have offered tremendous scope for researchers in this field and are fast

moving from concept to reality. Nanoparticles may be used for oral administration of gut-labile drugs or those with low aqueous solubility^[9]. These colloidal carriers have the ability to cross the mucosal barrier as such. In addition they have the potential for enhancing drug bioavailability via particle uptake mechanisms. Nanoparticulate oral delivery systems also have slower transit times than larger dosage forms that increases the local concentration gradient across absorptive cells, thereby enhancing local and systemic delivery of both free and bound drugs across the gut. These colloidal carriers are expected to develop adhesive interactions within the mucosa and remain in the gastrointestinal tract, while protecting the entrapped drug from enzymatic degradation, until the release of the loaded drug or their absorption in an intact particulate form. Most evidence suggests that the favored site for uptake is the Peyer's patches (PP) lymphoepithelial M cell. It has been shown that microparticles remain in the Peyer's patches, whereas nanoparticles disseminate systemically^[10]. It was therefore decided to prepare nanoparticles of acyclovir so as to optimize its delivery and overcome its inherent drawbacks^[11].

Polymeric nanoparticles have been used as potential drug delivery devices because of their ability to circulate for a prolonged period of time, target a particular organ, and their ability to deliver antiviral agents and genes^[12]. Polymeric nanoparticles have versatile potential for efficient exploitation of different drug delivery formulations and routes because of the properties provided by their small size. These possible benefits include controlled release, protection of the active pharmaceutical ingredient and drug targeting.

Polymeric nanodrug delivery systems will be prepared using different polymers such as polylactic acid/ polymethyl methacrylate / poly - ϵ - caprolactone / polylactide-coglycolide / gelatin / chitosan. Techniques like solvent evaporation / solvent displacement / interfacial deposition/salting out or emulsion/solvent diffusion will be evaluated to prepare polymeric nanoparticles. Nanoparticles prepared by above polymers and methods will have surface erosion or bulk erosion / biodegradation or swelling or combine pattern/ mechanism of drug release, which will be tailored to control drug release^[13].

Eudragit[®]RLPO, was selected as a polymer for preparation of acyclovir loaded nanoparticles due to its literature reported high permeability and application for sustained release drug delivery systems, using nano precipitation method^[14].

MATERIALS AND METHODS

Acyclovir was a gift sample from Ajanta Pharmaceuticals Limited, Mumbai, India; Eudragit[®]RLPO and Pluronic F68 were obtained as gift samples from Glenmark Pharmaceuticals Ltd. Nashik, India and StridesArco Lab, Bangalore, India, respectievely. Acetone and cellophane membrane were purchased from S.D. Fine Chem. Ltd., Mumbai, India and Hi-Media India respectively. All other reagents and chemicals used in this study were of analytical grade.

Formulation of Acyclovir loaded Eudragit® RLPO Nanoparticles:

Eudragit[®]RLPO nanoparticles were prepared by the nanoprecipitation method previously described by Fessi et al^[15] and adapted as follows: Organic phase was prepared by solubilizing Eudragit[®]RLPO in alcohol in an ultrasound bath for 10 min. Then, drug was dissolved in acidic solvent containing a hydrophilic surfactant (Pluronic[®] F68) at various concentrations, to prepare aqueous phase. This aqueous phase was poured into the organic solution drop wise, under magnetic stirring (500 rpm) for 2 h, thus forming a milky colloidal suspension. The organic solvent was then evaporated by using a rota evaporator. The resultant dispersion was dried using a freeze drying method.

Experimental Design:

The formulations were fabricated according to a 3^2 full factorial design, allowing the simultaneous evaluation of two formulation variables and their interaction. The experimental designs with corresponding formulations are outlined in Table 1. The dependent variables that were selected for study were, particle size (Y₁) and % drug entrapment(Y₂).

Determination of particle size

The particle size and size distribution of the acyclovir loaded Eudragit[®]RLPO nanoparticles were characterized by laser light scattering using particle size analyzer (Malvern Mastersizer Hydro 2000 SM, UK). The obscuration level was set between 7 to 11 %, distilled water was used as medium.

Morphological Analysis

The microscopic appearance of the Eudragit[®]RLPO nanoparticles was observed under optical and scanning electron microscopes (Jeol JSM-5410 LV, Tokyo, Japan). The scanning electron microscope photomicrographs were taken at 15 kV in various magnifications appropriate to each formulation.

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Table 1: Experimental Design And Parameters For 3 ² Full Factorial Design Batches					Batches
Batch	Variable	level in	Particle	% drug	% Free
Code	Coded	Form	size(nm)	entrapment	Drug
	\mathbf{X}_{1}^{*}	$\mathbf{X}_{2}^{\ \#}$	±SD**	±SD**	±SD**
F1	+1	+1	410±35.6	56.2±1.02	36.10±0.96
F2	+1	0	566 ± 54.9	66.8 ± 1.32	18.45 ± 0.04
F3	+1	-1	743±70.8	72.4±1.65	15.55 ± 0.03
F4	0	+1	189±21.2	57.5 ± 1.45	24.53 ± 0.86
F5	0	0	195±17.9	67.9±1.62	20.96 ± 0.06
F6	0	-1	256 ± 24.6	78.5±1.34	$5.9 {\pm} 0.01$
F7	-1	+1	74±6.98	48.6±1.52	43.5±1.02
F8	-1	0	128±11.6	62.4±1.64	22.4±0.81
F9	-1	-1	172±17.8	68.7±1.74	11.94 ± 0.03
С	-0.5	-0.5	169	66.6	13.64

**All the tests were carried out in triplicate. C check point batch. * Eudragit® RLPO (X₁) transformed levels in drug: polymer ratios are: -1= 1:1.5; '0'=1:2, +1=1:2.5, -0.5=1:1.75, # For surfactant (Pluronic F68) (X₂) transformed levels in % are: 1=0.25; '0'=0.5, +1= 0.75, -0.5=0.375

Determination of Encapsulation Efficiency

The free drug (per 100 mg of formulation) was estimated by taking said quantity of formulation in dialysis bag (cellophane membrane , molecular weight cut off 10000-12000 Da, Hi-Media, India) which was tied and placed into 100 ml 0.1N HCl on magnetic stirrer . At predetermined time intervals, 5 ml of the samples were withdrawn by means of a syringe. The volume withdrawn at each interval was replaced with same quantity of 0.1N HCl. The samples were analyzed for free drug by measuring the absorbance at 252 nm using UV-visible spectrophotometer (Shimadzu UV-1700) after suitable dilution. Above described process of withdrawing sample and analysis was continued till a constant absorbance was obtained^[16].

Encapsulated drug (per 100 mg of formulation) was estimated by taking residue formulation remained in dialysis membrane after estimation of free drug content, as described above. Quantity remained in dialysis membrane was added to ethanol (10 ml) to dissolve Eudragit[®]RLPO and filtered. Residue remaining on filter paper was dissolved in 100 ml of 0.1N HCl and after removing supernatant, sample was analyzed for drug content by measuring the absorbance at 252 nm using UV-visible spectrophotometer (Shimadzu UV-1700) after suitable dilution. The percentage of drug entrapped and the percentage of free drug are calculated by following equation^[17].

Statistical Analysis:^[18]

The results from factorial design were evaluated using PCP Disso 2000 V3 software. Step-wise backward linear regression analysis was used to develop polynomial equations for dependent variables particle size (Y_1) and % drug entrapment (Y_2) which bear the form of equation-1:

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_{12} X_1 X_2 + b_{11} X_1^2 + b_{22} X_2^2 - \dots - 1$$

Where Y is dependent variable, b_0 arithmetic mean response of nine batches, and b_1 estimated coefficient for factor X_1 . The main effects (X_1 and X_2) represent average result of changing one factor at a time from its low to high value. The interaction term (X_1X_2) shows how the response changes when two factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2 are included to investigate non-linearity. The validity of the developed polynomial equations was verified by preparing extra design check point formulation \mathbb{O} .

Drug Release Study:

A quantity of selected factorial formulations equivalent to

Formulae to calculate % free drug and % drug entrapment

Table 2: Various Parameters Determined For Drug Release From Nanoparticles Based On Peppas Model, Higuchi Model And Diffusion Profile						
Formulation	Correlation Coefficient (Zero order)	Correlation Coefficient (First Order)	Kinetic Exponent, N	Correlation Coefficient (Higuchi model r ²)		
F1	0.5997	0.8526	0.6201	0.9897		
F2	0.6911	0.8740	0.7146	0.9910		
F3	0.7884	0.9089	0.7992	0.9934		
F4	0.6113	0.9182	0.5765	0.9930		
F5	0.6532	0.9170	0.5919	0.9974		
F6	0.8338	0.9510	0.8701	0.9892		
F7	0.4395	0.9940	0.4557	0.9853		
F8	0.3077	0.8483	0.4578	0.9776		
F9	0.5641	0.8815	0.6453	0.9861		
Table 3: Parameters of Check point formulation						
	Predicted values Observed values					
Formulation	Particle Size (nm)	% drug entrapment	Particle Size (nm)	e % drug entrapment		
С	176.62	70.66	169	66.6		

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25 mg of the drug (Table 3 indicates drug content per 100 mg of formulation) was taken in the dialysis bag (cellophane membrane, molecular weight cut off 10000-12000 Da, Hi-Media, India). The dialysis bag was then suspended in a flask containing 100 ml of phosphate buffer (pH 7.4) on a magnetic stirrer at $37\pm0.5^{\circ}$ at 100 rpm. Required quantity (5 ml) of the medium was withdrawn at specific time periods (1, 2, 3, 4, 6, 8,10, 12, 24, 32 h) and same volume of dissolution medium was replaced in the flask to maintain a constant volume. The withdrawn samples were filtered and then 5 ml filtrate was made up to volume with 100 ml of phosphate buffer (pH 7.4). The samples were analyzed for drug release by measuring the absorbance at 252 nm using UV-visible spectrophotometer (Shimadzu UV-1700)^[19].

Drug-Polymer Interaction Studies:

Differential scanning calorimetry (DSC) is one of the most powerful analytical techniques, which offers the possibility of detecting chemical interaction. Acyclovir (pure drug), Eudragit®RLPO, and physical mixtures of drug and polymer at different ratios (1:1, 1:1.5, 1:2, 1:2.5) were kept at $40 \pm 2^{\circ}/75 \pm 5\%$ RH. Samples at 0,1,2,3 and 6 months were withdrawn and sent for testing. Also drug-polymer interaction study for selected formulation of prepared nanoparticles has been carried out with the same DSC technique.

RESULTS AND DISCUSSION

Out of all factorial formulations developed by the above described method, formulations F1, F2, F4, F5, F7, F8 and F9 were found to be free flowing i.e. non sticky but formulations, F3 and F6 were found to be sticky. All formulation were white and powdery in appearance.

The particle size affects the biopharmaceutical, physicochemical and drug release properties of the nanopaticles. A graphical representation of the particle size of Eudragit[®]RLPO nanoparticles obtained is given in Fig.1. Particle size is an important parameter, because it has a direct relevance to the stability of the formulation, also larger particles tend to aggregate to a greater extent compared to smaller particles, thereby resulting in sedimentation. The amount of stabilizer used has an effect on the properties of nanoparticles. If the concentration of stabilizer is too low, aggregation of the nanoparticles will take place, whereas, if too much stabilizer is used, drug incorporation could be reduced as a result of the interaction between the drug and stabilizer^[20].

The effect of the concentration of the polymers tested is negative or positive. A positive effect would imply that increasing the concentration causes the emulsion to have larger droplets, hence leading to larger particles. A negative effect means that increasing the concentration causes the emulsion to be more stable, hence leading to smaller particles^[20].

From Fig.2 and Table 1, it is revealed that as drug : polymer (acyclovir:Eudragit[®]RLPO) ratio increased from 1:1.5 to 1:2 particle size increased significantly and drug entrapment also increased but thereafter, further increase in drug:polymer ratio showed reduced or insignificant change in the drug entrapment efficiency.

This can be explained by observing drug entrapment efficiency of factorial formulations F3, F6, F9 where drug: polymer ratio increased from 1:1.5 to 1:2 and 1:2.5 respectively with constant concentration of stabilizer (Pluronic F68) i.e. 0.25%. Drug entrapment efficiency increased from 72.4% to 78.5% and then decreased to 68.7 %. It is also observed that as percentage of stabilizer increased from 0.25% to 1%, entrapment efficiency and particle size decreased significantly, the same can be explained with respect to factorial formulations F1, F4, F7 and F2, F5, F8 where it is observed that as drug: polymer ratio increases, entrapment efficiency increased significantly but further increases in drug: polymer ratio has negative or insignificant effect on drug entrapment. For factorial formulations F1, F2, F3 where drug:polymer ratio is constant i.e. 1:2.5 and concentration of stabilizer decreased from 0.75 % to 0.25%, drug entrapment efficiency increased from 56.2% to 72.4% and particle size increased from 410 nm to 743 nm. Thus it can be concluded that the surfactant had greater influence on both dependent parameters (particle size and drug entrapment) as compared to drug: polymer ratio.

Drug release from nanoparticles and subsequent biodegradation are important for developing successful formulations. The release rate of nanoparticles depends upon i) desorption of the surface-bound/adsorbed drug; ii) diffusion through the nanopaticle matrix; iii) diffusion (in case of nanocapsules) through the polymer wall; iv) nanoparticle matrix erosion: and v) a combined erosion/diffusion process. Thus, diffusion and biodegradation govern the process of drug release^[21].

It is proposed that there are two main pathways of drug release from swellable nanoparticles. First, there is a rapid release of drug from the nanoparticles, which could stem from drug adsorbed on the nanoparticle surface. Then a slower, more controlled release takes place, which is possibly related to nanoparticle diffusion of the drug through the nanoparticle shell^[22].

The mechanism of drug release from nanoparticles is determined by different physical-chemical phenomena. The exponent 'n' has been proposed as indicative of the release mechanism. In this context, n = 0.43 indicates Fickian release, n = 0.85 indicates a purely relaxation (Case II) and > 0.85 indicates super case II controlled delivery. Intermediate values 0.43 < n < 0.85 indicate an anomalous behavior (non-Fickian kinetics) corresponding to coupled diffusion/polymer relaxation^[23].

The average percentage release was fitted into different release models: zero order, first order and Higuchi's square root plot. The models giving a correlation coefficient close to unity were taken as the order of release In vitro drug release data of all selected factorial formulations was subjected to goodness of fit test by linear regression analysis according to zero order and first order kinetic equations, Higuchi's, and Korsmeyer-Peppas models to ascertain the mechanism of drug release. From various parameters determined for drug release from nanoparticles based on Peppas model, Higuchi model and diffusion profile (Table No.2), it is evident that values of 'r²' for Higuchi plots of factorial formulations ranging from 0.9776 to 0.9934, for first order plots 0.8483 to 0.9940 and those of 'n' (diffusion exponent) values of Peppas equation from 0.4557 to 0.8701. This data reveals that drug release follows first order release kinetics with non-Fickian diffusion mechanism for all factorial formulations except F6. where drug release follows first order release kinetics with case II diffusion mechanism. Finally, it can be concluded that the different drug release rates may be attributed to different sizes of the nanoparticles. It is expected as the particle size of Eudragit[®]RLPO nanoparticle is smaller, their surface area will be more and the drug release is faster.

As prepared polymeric nanoparticles has size range in between 74 nm to 566 nm, for these colloidal carriers sites for uptake are expected as Peyer's patches (PP) and lymphoepithelial M cell. It has been shown that larger nanoparticles remain in the Peyer's patches, whereas nanoparticles with lower particle size range disseminate systemically^[10].

Development of Polynomial Equations:

From the data of experimental design and parameters (Table No.1) for factorial formulations F1 to F9, polynomial equations for two dependent variables (particle size and % drug entrapment) have been derived using PCPDisso 2000V3 software.

The equation derived for particle size is:



















 $Y_1 = 213.333 + 224.16X_1 - 83.00X_2 + 135.5X_1^2 - 2$

The equation derived for % drug entrapment is:

In equations (2) negative sign for coefficient of X_2 indicates that the particle size of nanoparticles increases when concentration of stabilizer (Pluronic F 68) is decreased and positive sign for coefficient of X_1 indicate positive effect of polymer concentration (Eudragit[®] RLPO) on particle size.

In equation (3) positive sign for coefficient of X_1 indicates

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that the % drug entrapment increases when concentration of polymer (Eudragit[®]RLPO) increases and negative sign for coefficient of X_2 indicates that % drug entrapment of nanoparticles increases when concentration of stabilizer (Pluronic F 68) decreases.

Validity of the above equations was verified by designing check point formulation (C). The particle size and % drug entrapment predicted from the equations derived and those observed from experimental results are summarized in Table No.3.The closeness of predicted and observed values for particle size and % drug entrapment indicates validity of derived equations for dependent variables.

Response Surface Plots:Graphical presentation of the data can help to show the relationship between response and independent variables. Graphs gave information similar to that of the mathematical equations obtained from statistical analysis. The response surface graphs of particle size and % drug entrapment are presented in fig 3 and fig. 4 respectively.

The response surface plots illustrated that as concentration of polymer (Eudragit[®]RLPO) increases, the value of dependent variable, i.e. particle size increase and as concentration of stabilizer (Pluronic F 68) increases the value of dependent variable, i.e. particle size decreases. Similarly the response surface plots for % drug entrapment shows positive effects of independent variable, i.e. polymer concentration (Eudragit[®]RLPO) and negative effect of other independent variable, i.e. concentration of stabilizer (Pluronic F 68).

DSC gives information regarding the physical properties like crystalline or amorphous nature of the samples. The DSC thermogram of acyclovir (Fig 5a) shows an exothermic peak at 267.03° corresponding to its melting temperature , which was not detected in the thermograms for acyclovir loaded Eudragit[®]RLPO nanoparticles (Fig 5b). It has been shown by a couple of authors that when the drug does not show its exothermic peak in the formed nanoparticles, it is said to be in the amorphous state^[20]. Hence it could be concluded that in the prepared acyclovir loaded Eudragit[®]RLPO nanoaprticles, the drug was present in the amorphous phase and may have been homogeneously dispersed in the polymer matrix.

CONCLUSION

Acyclovir loaded Eudragit[®]RLPO nanoparticles were prepared by the nanoprecipitation method. The application of factorial design gave a statistically systematic approach for the formulation of nanoparticles with desired particle size and high entrapment efficiency. Nanoparticles, with smallest particle size, up to 74 nm and highest drug entrapment efficiency, up to 74.5% has been achieved using Eudragit[®]RLPO polymer with nanoprecipitation method. Drug: polymer ratio and concentration of surfactant were found to influence the particle size and entrapment efficiency of acyclovir loaded Eudragit®RLPO nanoparticles. In vitro drug release study of factorial formulations showed release in 32 h in the range 67.928% to 97.957% . The release was found to follow first order release kinetics with non-Fickian diffusion mechanism for all batches except F6 where drug release follows first order release kinetics with case II diffusion mechanism.It is expected that these particulate nanodrug delivery systems will very well uptaken by Pever's patches (PP) and disseminate systemically due to their particle size and polymer nature. These preliminary results indicate that acyclovir loaded Eudragit®RLPO nanoparticles could be effective in sustaining drug release for a prolonged period.

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