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MESSAGE FROM SECRETARY

It is a matter of great happiness to me to know that student and faculty of Geethanjali are bringing out an excellent piece of scientific and technical magazine “PHARMAGAZINE”. As I understand , this demonstrates the literary, art, imaginative, technical and scientific skills of our students and faculty.

My heartfelt congratulations to the entire team for keeping the tradition high and raising the bar of the presenting the articles. Keeping this in mind. I expect the contributions to this magazine to be of very high standard and quality.

I Wish all the success for this venture.

G.R.RAVINDER REDDY
M.Tech (NIT), Ex IPS
Secretary
Geethanjali College o Pharmacy

MESSAGE FROM PRINCIPAL

Spell bound by the efforts that our students and faculty have shown, I take immense pleasure appreciating them for coming up with the first edition of the technical and scientific magazine. Their enthusiasm has enraptured me and took me back to my Pharmacy days.

No child is a congenital Einstein or Newton. In fact, it is through one's consistent hard work and passion to do something that takes one to glorious heights.

Hope the scientific and technical Magazine being published stands elite among the other magazines published in some of the colleges apparently to spread knowledge and help students to get acquainted with the state of the art of science and technology. It even helps in developing one's leadership skills and team spirit. I congratulate all of them for their untiring efforts and would advise the students to utilize this opportunity. My best wishes to the editorial members of the scientific and technical Magazine "PHARMAGAZINE" and hope to see it flourishing , inculcating creativity and innovation in the pursuit of spreading knowledge.

Dr. M. RAVI KUMAR
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PREPARATION AND EVALUATION OF RIZATRIPTAN SUBLINGUAL TABLETS BY USING SUPERDISINTEGRANTS

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ABSTARCT

The present study is aimed to formulate and evaluate Sublingual tablets of Rizatriptan**.** Sodium starch glycollate, Gellan gum, and Croscarmellose sodium were employed as super disintegrating agents to increase the solubility and dissolution rate of the rizatriptan drug molecule. The direct compression method involves in the preparation of sublingual tablets using 8 station rotary punching machine with a 6mm punch for all formulations. The mixture(blend) of all the formulations were passed through precompression parameters like angle of repose, bulk density and tapped density which shows good flow property of powders. The post-compression parameters were implemented for the prepared tablets and they pass all the quality control parameters as per IP limits. The FTIR and DSC studies were analyzed for drug and excipient studies. The F4 formulation is considered as the optimized formulation as it showed that maximum % drug release i.e.99.16% in 8 min. The F4 formulation contains Gellan gum as super disintegrate in the concentration of 10mg.

Keywords: Rizatriptan, Croscarmellose sodium, Gellan gum and sodium starch glycolate.

M.Geetha, Dr.T.Mangilal, Dr.M.Ravi Kumar,J.Naveen, Preparation and evaluation of rizatriptan sublingual tablets by using superdisintegrants, European Journal of Biomedical and Pharmaceutical sciences,2018, 5 (9), 2349-2887 .

1. INTRODUCTION

Sublingual administration of drugs shows the fast onset of action is carryout as compared to the oral route .The retention time of sublingual medication is 3 to 10 times greater than the oral route and is just passed by the hypodermic infusion method. Sublingual route has several advantages over the avoidance of first-pass metabolism, progressed patient compliance and ease of self-medication. This course has particular points of interest over the enteral and parenteral course of

medication because of its high blood supply, the onset of action and improved bioavailability into the systemic circulation[1]. Various components like pH, molecular weight, and lipid solubility may exploit this technique. From these properties, a solvent medication may disperse too gradually through the mucosa to be dynamic. The sublingual glands are also considered as salivary glands which produce the mucin and helps in the fabrication of saliva, required for the breakdown of particles. These glands present in the lining of the oral cavity that is below the tongue. This also provides slippery that helps in chewing and swallowing the food. The amount of drug that reaches into the systemic circulation from the site of administration is directly proportional to membrane thickness. It is expressed in the following order Sublingual>buccal>gingival>palatal[2].

Because of greater permeation and high blood supply, this release rapid onset of action and instant dosing regimen with less delivery period of drugs with the sublingual route. Sublingual means "under the tongue". It is considered to a method of placing drug via mouth so that the drug highly absorbed through blood vessels below the tongue more than digestive track. The sublingually administered drug pharmacologically activates in 1 – 2 minutes which is effectively impressed in this route. Some of the drugs which are administered through the sublingual route are Steroids, barbiturates, cardiovascular drugs, and enzymes.These administered drug directly reaches to nutritional benefits which avoid subject to gastric system and liver[3].

1.1Factors Affecting the Sublingual Absorption[4,5]

1. Solubility in Salivary Secretion
2. Binding to Oral Mucosa
3. pH and pKa of The Saliva
4. Lipophilicity of Drug
5. Thickness of Oral Epithelium.

Rizatriptan is structurally and pharmacologically related to other selective 5-HT 1B/1D receptor agonists and has only a weak affinity for 5-HT_{1A}, 5-HT_{5A}, and 5-HT_7 receptors and no significant affinity or pharmacological activity at 5-HT_2, 5-HT_3 or 5-HT_4 receptor subtypes. Rizatriptan is used to treat migraines. It helps to relieve a headache, pain, and other migraine symptoms including nausea, vomiting, sensitivity to light/sound. The migraines can be easily treated to return your normal routine and decreases the pain medications. And also it relieves the pain caused by certain nerves in the brain[6].

2. MATERIALS AND METHOD

2.1 Materials

Rizatriptan is a gift sample from Aurobindo Pharma Ltd., Hyderabad, Microcrystalline cellulose from Signet chemical corp, Sodium starch glycolate from Aurobindo Pharma Ltd., Hyderabad ,

Gellan gum from Aurobindo Pharma Ltd., Hyderabad, Croscarmellose sodium from Signet Chemical Corp, Magnesium stearate from S.D.Fine Chem Ltd, Talc from S.D. Fine Chem Ltd.

2.2 Method

Preparation of sublingual tablets is done by using direct compression methods for nine formulations were prepared at different ratios. [7, 8]

All the formulations were weighed and the required quantity of the drug and excipients are mixed thoroughly in a polybag. The blend is compressed by using rotary tablet machine-8 station with 6mm flat punch, B tooling. Each tablet contains 10 mg of Rizatriptan and other pharmaceutical ingredients. Total weight of the tablet was found to be 150 mg. The composition of various formulations as shown in the table no.1

Table no.1 Composition of F1 to F9 formulations

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Rizatriptan	10mg	10mg	10mg	10mg	10mg	10mg	10mg	10mg	10mg
Croscarmellose Sodium	10mg	15mg	20mg	-	-	-	-	-	-
Gellan Gum	-	-	-	10mg	15mg	20mg	-	-	-
Sodium Starch Glycolate	-	-	-	-	-	-	10mg	15mg	20mg
Magnesium Stearate	3mg	3mg	3mg	3mg	3mg	3mg	3mg	3mg	3mg
Talc	3mg	3mg	3mg	3mg	3mg	3mg	3mg	3mg	3mg
Mcc	124mg	119mg	114mg	124mg	119mg	114mg	124mg	119mg	114mg
Total Weight	150mg	150mg	150mg	150mg	150mg	150mg	150mg	150mg	150mg

2.3EVALUATION TESTS

2.3.1Precompression parameters[9,10]

Precompression parameters were studied before punching a tablet.

2.3.1.1 Bulk density: It is the ratio of the known mass of the powder sample which is untapped and its volume including interparticulate void volume.

$$\text{Bulk density} = \frac{\text{Mass of the powder}}{\text{Bulk voume of the powder}}$$

It is measured in gm/ml.

2.3.1.2 Tapped density: It is the ratio of the mass of the powder to the volume occupied by the powder after a fixed number of taps.

$$\text{Tapped density} = \frac{\text{Mass of the powder}}{\text{Tapped volume of the powder}}$$

It is measured in gm/ml.

2.3.1.3 Compressibility index

$$\text{carrs index\%} = \frac{\text{tapped density} - \text{Bulk density}}{\text{Tapped density}} \text{ x } 100$$

2.3.1.4 Hausner's Ratio

$$\text{Hausner Ratio} = \frac{\text{tapped density}}{\text{bulk density}} \text{ x } 100$$

2.3.1.5 Angle of Repose: It is the minimum angle at which any piled up bulky or loose material stands without falling down. The angle of repose ranges from 0^0 to 90^o.

The tangent angle of repose (θ) was calculated by an equation,

Tan θ = h/r

Angle of repose (θ) = tan-1(h/r)

Where,

h=height of the pile, r=radius of the pile, θ=angle of repose.

2.3.2 Drug- Excipient Compatibility Studies[11,12]

2.3.2.1 FT-IR (Fourier Transform Infrared): FT-IR study was carried out to find out the compatibility between the pure drug of rizatriptan and polymers such as gellan gum, sodium starch glycolate, and crosscarmellose sodium. The prepared tablet was kept on the sample holder and scanned between the range of 4000cm^{-1} to 400cm^{-1}. The amount of rizatriptan loaded into sublingual formulation was analyzed in FT-IR. The spectra obtained was compared and interpreted for the functional group peaks.

2.3.2.2 Differential Scanning Colorimetry: The molecular state of the drug was evaluated by performing a DSC analysis of Rizatriptan and optimized formulations. The sample was heated in hermetically heated aluminum pans between the ranges of 35°C-350°C at a constant rate of 10.0°C/min under a nitrogen purge at 20ml/min.

2.3.3 Post compression parameters[13,14]

2.3.3.1 Weight variation: Variations in weight were tested in randomly selected 20 different tablets from every batch. Digital electronic balance (Citizen CTG-302, India) is used for measuring weight variations. Then individual tablets were weighed and compared with an average weight. Weight values were reported in mg. Mean and SD were calculated.

2.3.3.2 Hardness (or) tablet crushing strength: The resistance of the tablets to capping, abrasion or breakage during storage, transportation and handling before usage depends on its hardness. Tablet hardness is the amount required to crush or fracture a tablet kept on its edge. Monsanto type (Make: Singhala) hardness tester is used for testing hardness. This instrument measures the crushing strengths.

It is measured in kg/cm^2.

2.3.3.3 Thickness: The diameter and thickness of the tablets were measured for 20 tablets from each formulation. Digital Vernier caliper is used for this study and it gives accurate measurements and information about variation between tablets. It is measured in mm.

2.3.3.4 Friability (F): Friability of the tablet is determined using Roche friabilator. Preweighed 20 tablets were subjected to the combined effect of abrasion and shock in a Friabilator containing a plastic chamber revolving at 25 rpm up to 100 revolutions. Remove the dust using soft muslin cloth, and then tablets were re-weighed and friability percentage was calculated using the following formula.

$$\%\text{Friability} = \frac{\text{Tablet weight before friabilty} - \text{Tablet weight after friability}}{\text{Tablet weight before friabilty}} \text{ x } 100$$

2.3.3.5 Wetting time [15]: The initial process in the disintegration of sublingual tablets involves water uptake and wetting of the tablet. So the determination of wetting time is also important. A Petri-dish containing 6 ml of the distilled water is taken and tissue paper folded twice is placed in it. The time required for the upper surface of the tablet to become complete wet is the wetting time.

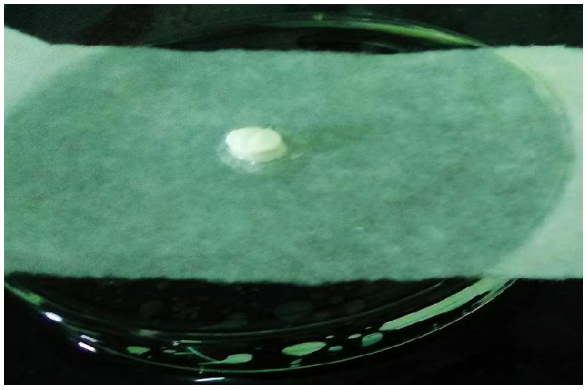


Figure no. 1 wetting time of sublingual tablet (F4)

2.3.3.6 Water absorption ratio[15]: A pre-weighed tablet (W_1) is placed in a Petri dish in the similar way as described in the wetting time test, the tablet has absorbed water completely, it is removed and weight is noted (W_2). Water absorption ratio R is calculated as

$R = W_2 - W_1/W_1 \times 100$

Where, W_1 = Weight of the tablet before immersing in water

W_2= Weight of the tablet after immersing in water

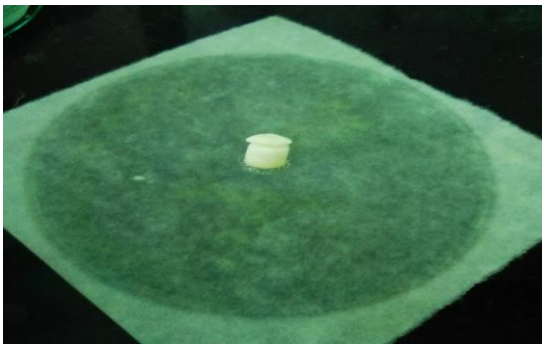


Figure no. 2 Water absorption ratio of sublingual tablet (F4)

2.3.4 *Invitro* dissolution studies[16]

In-vitro release studies were carried out using a modified USPII dissolution test apparatus (Lab India, DS-800).

The dissolution medium was filled with 500ml of phosphate buffer of pH 6.8 at a speed of 50rpm at 37^0c were used in each test. Samples of dissolution medium (5ml) were withdrawn for every 2min and assayed for Rizatriptan by measuring the absorbance at 259 nm. For all tests 5ml of the test sample were collected at specified time periods and replaced with the same volume of phosphate buffer pH 6.8.

3. RESULTS AND DISCUSSION

3.1 Preformulation studies

3.1.1 Spectrophotometric Studies

Standard Calibration curve of Rizatriptan

Table no.2 Concentration and absorbance obtained for calibration curve of Rizatriptan in pH 6.8 phosphate buffer

S. No.	Concentration (µg/ml)	Absorbance* (at 259 nm)
1	0	0±0
2	0.5	0.1599±0.0003
3	1	0.3079±0.0003
4	1.5	0.4507±0.0002
5	2	0.6029±0.0004
6	2.5	0.7349±0.0002

***All values are the mean of six readings± SD**

It was found that the estimation of Rizatriptan by UV spectrophotometric method at λ_{max} 259nm in pH 6.8. Phosphate buffer had good reproducibility and this method was used in this study. The correlation coefficient of the standard curve was found to be closer to 1, at the concentration range, 2- 10µg/ml. The regression equation generated was y = 0.2941x+ 0.0085, R^2 = 0.9994.

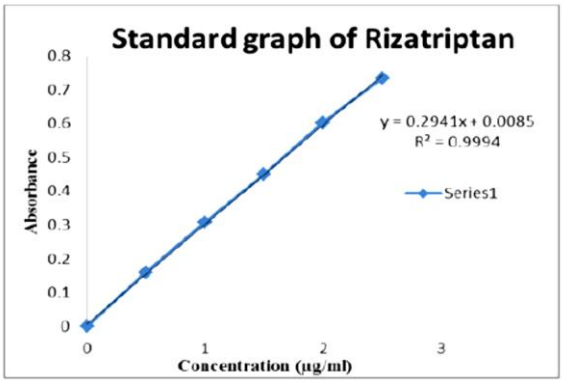


Figure no. 3 Standard graph of Rizatriptan in pH 6.8 phosphate buffer

3.2 Compatibility Studies of Rizatriptan Sublingual Tablets

3.2.1Fourier Transform Infrared Spectroscopy

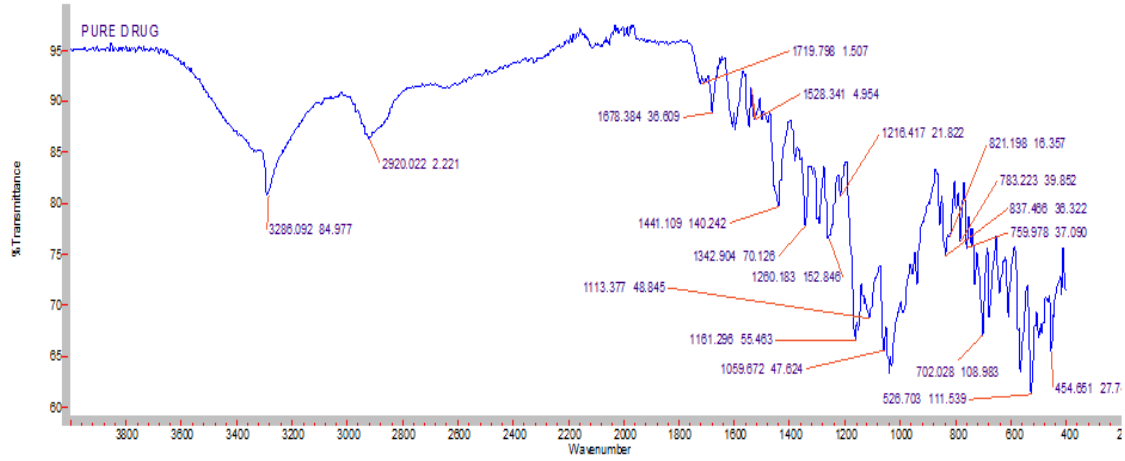


Figure no. 4 FTIR Spectrum of pure drug

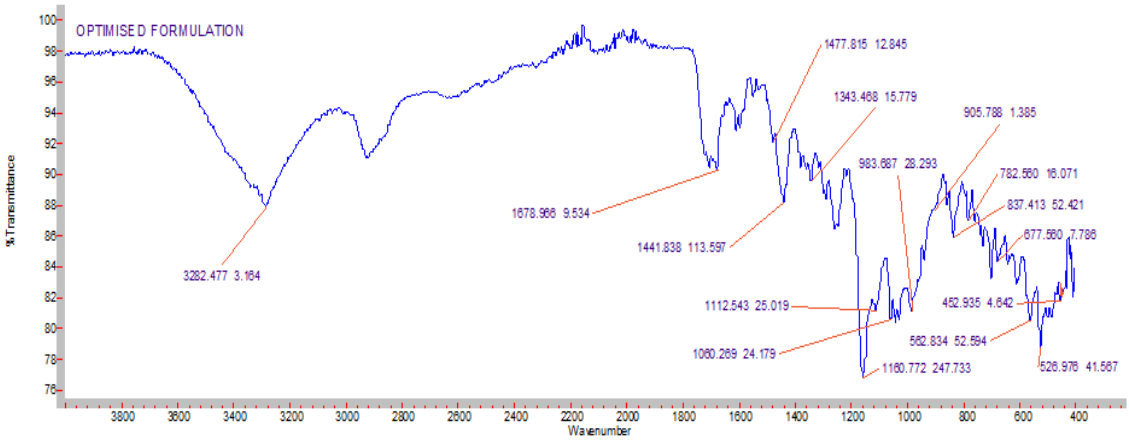


Figure no.5 FTIR spectrum of optimized formulation

Table no.3 Frequency ranges of Rizatriptan (Pure drug) and Optimized formulation

S. No.	Frequency of Pure Drug	Frequency of Optimized Drug	Frequency Range	Functional Group
1	3286.092	3282.477	3600-3200	Alcohol O-H stretch
2	1678.384	1678.966	1680-1600	C=C alkene
3	1528.341	1477.815	1600-1400	C=C aromatic
4	1441.109	1441.838	1480-1440	CH_2 bend
5	1342.904	1348.488	1400-1300	NO_2 stretch
6	1113.377	1112.543	1400-1000	C-F

The FT-IR of Rizatriptan exhibits reported peak values in the range of Pharmacopeial limit. The FT-IR of a mixture (rizatriptan +excipients) exhibits the reported values within the FTIR

frequency ranges. From the report of FTIR data, it was concluded that there is no interaction between the rizatriptan pure drug and mixture (rizatriptan+excipients). As shown in table no.3.

3.2.2 Differential scanning colorimeter

Figure no. 6 Differential scanning colorimeter spectrum of Rizatriptan

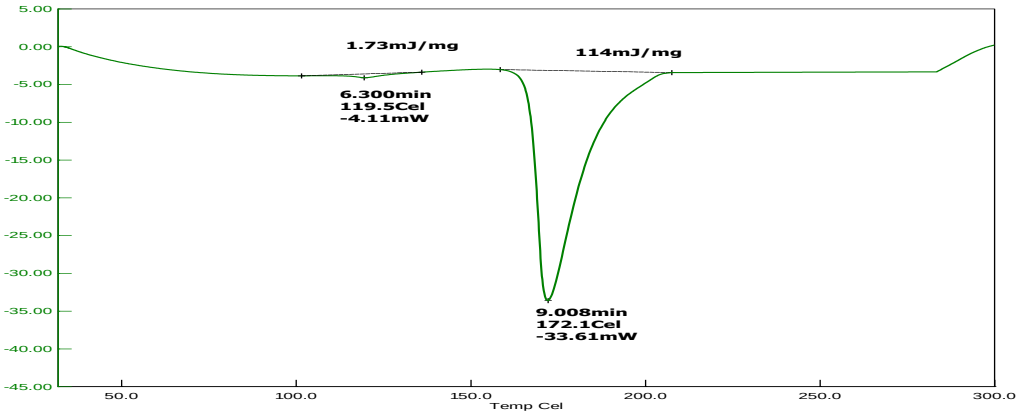
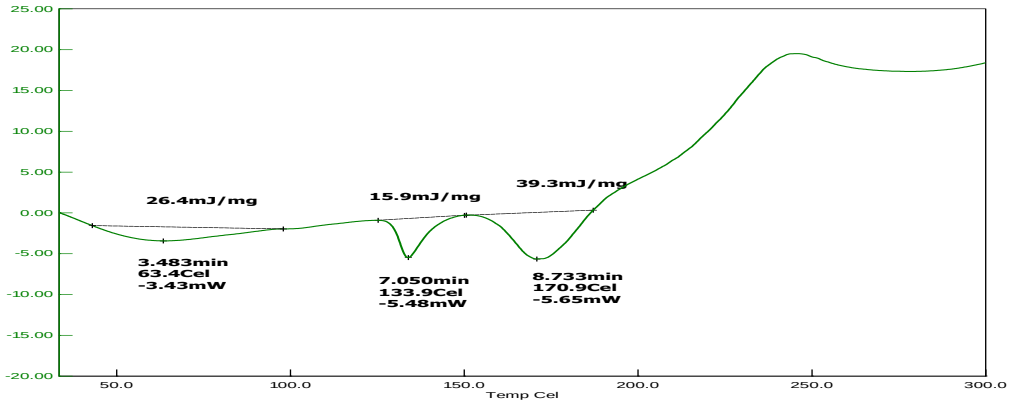


Figure no. 7 DSC of optimized formulation (F4)

Pure ams show very t 170.9ºC. DSC ence there was r



3.3 P

Table no.4 Pre-compression parameters

Formulations	Bulk Density (gm/cm^2)*	Tap Density (gm/cm^2)*	Carr's Index (%)*	Hausner ratio*	Angle Of Repose(Θ)*
F_1	0.45±0.0264	0.55±0.0360	18.18±0.0458	1.22±0.0700	27.91±0.4373
F_2	0.47±0.0173	0.55±0.0482	14.54±0.0529	1.17±0.1062	28.23±0.5383
F_3	0.50±0.0300	0.58±0.0655	13.79±0.0300	1.16±0.0902	29.34±0.9651
F_4	0.46±0.0264	0.55±0.0400	16.36±0.0378	1.19±0.1108	26.71±1.0215
F_5	0.50±0.0458	0.58±0.0360	13.79±0.0416	1.16±0.0902	29.34±0.9888
F_6	0.47±0.0346	0.55±0.0346	14.54±0.0529	1.17±0.0820	28.23±0.8972
F_7	0.50±0.0435	0.58±0.0208	13.79±0.0100	1.16±0.0163	29.34±2.1704
F_8	0.41±0.0100	0.50±0.0264	18±0.180278	1.21±0.1202	26.78±1.4696
F_9	0.48±0.0360	0.57±0.0529	17±0.0500	1.19±0.0404	26.7±0.0600

***All values are the mean of three readings± SD**

The data were shown in Table 4.The values for angle of repose was found in the range of 25°-30°. The bulk densities and tapped densities of various formulations were found to be in the range of 0.41 to 0.50 (gm/cc) and 0.50 to 0.58 (gm/cc) respectively. Carr's index of the prepared blend falls in the range of 13.06% to 18.18%. The Hausner's ratio falls in range of 1.14 to 1.22. From the result, it was concluded that the powdered blends have good flow properties and these can be used for tablet manufacture.

3.4 Post-compression parameters

Table no.5 Post compression Parameters

Formulation code	Weight variation (mg)*	Hardness (kg/cm^2)*	Thickness (mm)*	Disintegration Time (sec)*	Friability (%)*
F1	150±1.6329	2.5±0.0816	2.34±0.9579	60±0..8164	0.43±0.0941
F2	152±4.3204	2.6±0.1414	2.24±0.0748	62±1.6329	0.34±0.1423
F3	149±2.1602	2.5±0.3265	2.29±0.0927	72±2.1602	0.49±0.0697
F4	151±4.966	2.6±0.1632	2.28±0.2141	69±0.8164	0.47±0.0668
F5	152±3.2659	2.3±0.2449	2.39±0.0941	70±0.4082	0.49±0.2624
F6	153±3.7416	2.7±0.3559	2.24±0.2785	62±1.6329	0.34±0.0848
F7	152±4.5460	2.5±0.1632	2.29±0.1930	70±0.8164	0.49±0.0535
F8	150±6.5319	2.6±0.4320	2.36±0.8309	67±2.4494	0.34±0.2135
F9	152±5.8878	2.5±0.2943	2.26±0.2039	67±2.0548	0.34±0.2006

***All values are the mean of six readings± SD**

Table no.6 Post compression parameters of Rizatriptan sublingual tablets

Formulation Code	Wetting time(sec)*	Water absorption ratio (%)*	Assay (%)*
F1	1.03±0.00816	4.16±0.0326	97.23±0.8339
F2	1.04±0.01632	7.48±0.0902	98.55±0.4546
F3	1.15±0.02160	8.0±0.8164	98.16±0.8659
F4	1.29±0.00816	8.60±0.1632	99.24±0.8779
F5	1.50±0.01632	5.44±0.0588	98.16±0.3265
F6	1.59±0.02162	4.69±0.0294	98.55±0.9227
F7	1.05±0.00816	5.55±0.0216	98.16 ±0.6860
F8	1.02±0.01632	6.20±0.0432	99.25±0.2118
F9	1.08±0.03265	8.84±0.1423	99.25±0.1061

***All values are the mean of six readings ±SD**

Weight variation test: Weight variation test for all formulations was checked and the difference between weight variation and the percent deviation was checked for each tablet and was shown in table no 5. The average weight of tablet ranges from approximately 98 to 150.5mg. So, the permissible limit is ±10% (=100mg). It concluded that the tablet weights are within the pharmacoepial limit.

Hardness test: The hardness of three tablets of each batch was checked by using Monsanto hardness tester and the data's were shown in Table no 5. The results showed that the hardness of tablet falls in the range of 2.5 to 3.00 kg/cm^2, which were within IP limits.

Thickness: The Thickness of all formulations of three tablets was checked by using vernier caliper and data's were shown in table no 5. The result has shown that the average thickness of tablet ranges between1.56 to 1.64.

Friability: Percentage friability of each batch was evaluated for tablet and the data was shown in the table no 5.The average friability of all formulations lies in the range of 0.30.to 0.51%. It indicates a good mechanical resistance of tablets as per the official requirement of IP.

***Invitro* disintegration time**: Tablets of each batch were evaluated for *invitro* disintegration time and the data were shown in Table 5. The results showed that the disintegration time of prepared tablets was in the range of 12.66 to 30.33 seconds.

Wetting time: Tablets of each formulation were evaluated for wetting time and the data was shown in table no 6. The average wetting time of all formulations ranges between 1.02 to 1.54 seconds.

Water absorption ratio: The tablets of each formulation were evaluated for water absorption ratio and the data was shown in Table no 6. The result showed that water absorption ratio lies within the range of 4.40 to 8.84%.

Assay: Assay studies were performed for the prepared formulations and data's were shown in table 6. From assay studies, it was concluded that all formulations were showing the % drug content values within the range of 97.23 -99.2%.

***3.5 InVitro* Dissolution studies:** *Invitro* dissolution studies were carried out by using 500ml of pH 6.8 Phosphate buffer in USP dissolution apparatus by using paddle method. The dissolution studies

were carried out for about 30 min.

Table no.7 *In-vitro* Dissolution studies of all Rizatriptan sublingual tablets

Time(min)	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)	F7 (%)	F8 (%)	F9 (%)
1	15.46±3.0496	10.83±1.1834	49.72 ± 0.7657	24.37±0.4030	31.73±0.9354	18.35±0.7266	28.45±0.9483	39.50±0.5115	12.51±0.4972
2	26.63±5.7052	26.72±4.5334	60.16± 0.7216	31.68±0.5921	34.56±0.9064	22.9±1.2083	35.28±0.7257	46.35±0.7505	26.38±1.2458
3	35.64±8.6661	36.16±0.7858	68.15±2.1475	49.37±0.6394	41.91±0.7968	38.71±1.2668	48.90±0.6963	56.28±0.7654	35.17±0.7970
4	40.38±4.0150	47.46±5.2198	72.56±0.7858	58.35±0.6491	62.48±1.124	50.16±0.8334	66.83±0.8573	69.71±0.8591	47.37±0.5848
5	46.44±6.98	58.57±1.5346	78.41±1.4697	74.37±0.7141	69.3±0.8258	64.32±1.3383	78.17±0.5463	76.26±0.8153	54.96±1.4012
6	53.64±1.9142	68.25±2.8884	83.27± 1.2947	88.18±1.3465	75.49±1.2706	76.42±0.1451	82.45±0.1643	80.14±1.3934	62.56±0.3080
7	69.82±8.4175	73.19±2.1485	87.45± 0.5242	94.65±0.7406	82.33±0.7826	80.14±0.8386	87.16±0.8659	85.26±0.3709	78.35±1.3684
8	80.56±17.968	90.16±2.4986	94.26± 0.4819	99.16±0.5953	89.65±0.6312	92.46±0.1000	92.18±0.7061	91.28±0.077	89.26±0.2698

3.5.1 *Invitro* dissolution Studies of all formulations

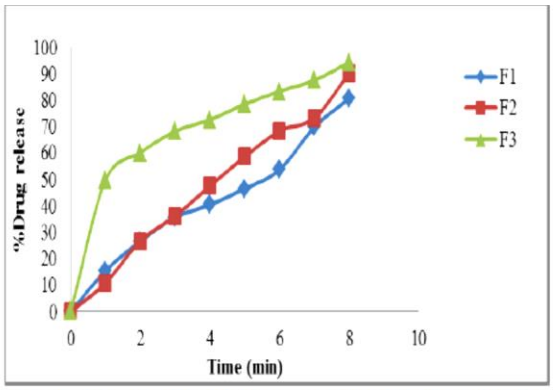


Figure No. 8 Dissolution profile of formulations prepared with Sodium starch glycollate as super disintegrate.

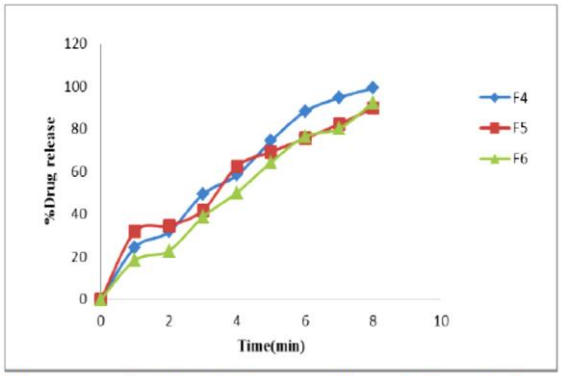


Figure no.9 Dissolution profile of formulations prepared with Gellan gum as super disintegrate.

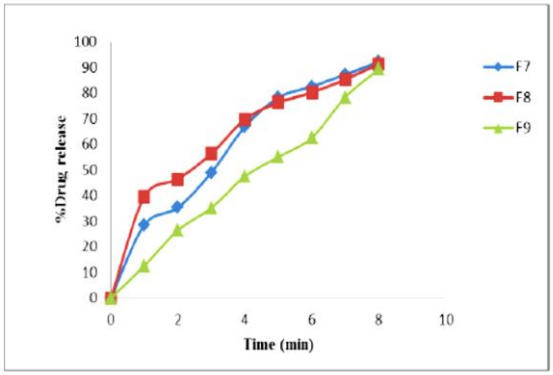


Figure. No. 10. Dissolution profile of formulations prepared with Croscarmellose sodium as super disintegrate.

From the table no.7 it was evident that the formulations prepared with super disintegrates,

the gellan gum showed maximum % drug release in 8 min i.e. 99.16% (F4 formulations and concentration of super disintegrate was 10 mg). So, the principle of super disintegrate was found to be useful to produce Sublingual tablets. The F4 formulation was considered as optimized formulation.

4. CONCLUSION

In the recent work, an attempt has been made to develop Sublingual tablets of Rizatriptan. Sodium starch glycollate, Gellan gum, and Croscarmellose sodium were employed as super disintegrating agents to enhance the solubility and dissolution rate of a selected drug molecule. Above all the formulations F4 formulation showed a maximum % drug release i.e., 99.16 % in 8 min hence it is considered as optimized formulation. The F4 formulation contains Gellan gum as super disintegrate in the concentration of 10 mg.

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PREPARATION AND *INVITRO* EVALUATION OF HIGHLY POROUS GASTRORETENTIVE FLOATING BECLOMETHASONE DIPROPIONATE TABLETS

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ABSRTACT

The present study is aimed to formulate floating gastro retentive (GR) tablets containing beclomethasone dipropionate using a sublimation material for prolongation of gastric residence time .Three different ratios of hydroxyl propyl methyl cellulose (HPMC) K4M is used in three different methods for the preparation of tablets. In this case, the drug release from tablet was highly dependent on the polymer concentrations. Camphor, the sublimation material is used in the preparation of GR tablets. Camphor changes to pores in the tablet during the sublimation process. As the camphor gets sublimed, floating properties and density of tablets were affected by the sublimation of camphor. Gastro retentive floating beclomethasone dipropionate tablets which were prepared floated for over 24 hrs and had no floating lag time. Therefore, as the concentration of camphor in the tablet matrix increases, the hardness of the tablet decreased after sublimation. Release profiles of the drug from the GR tablets were not affected by tablet density or porosity.

KEY WORDS: Beclomethasone Dipropionate, gastro retentive floating tablets, HPMCK4M, sublimation method.

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INTRODUCTION

The principle and more advisable route for delivering a drug is the oral route, but in case of physiological variability like gastro intestinal transit and GRT there is a major problem. The controlled oral drug delivery of GRT is always less than 12h, and it plays a vital role in complete dosage form transit [1,3]. These characteristics lead to evolution of a drug delivery system that retains in the stomach for a prolonged and predictable time[2].

Floating drug delivery systems (FDDS) have low bulk density than that of gastric fluids. Due to their lower densities, FDDS float above the gastric content without effecting gastric emptying rate for longer duration of time and it provides controlled release of drug [3]. These systems have been

extensively used because there are no interactions in relation to the motility of the GIT and a large number of floating dosage forms commercialized and marketed worldwide. Two systems have been used in the development of FDDS, on the basis of mechanism of buoyancy. They are effervescent systems and non effervescent system In Effervescent systems effervescent substances like carbonate/ bicarbonate salts and citric / tartaric acids are used to liberate Co_2. The liberated Co_2 is entrapped in the jellified hydrocolloid layer of the systems thus specific gravity is decreased and it is made to float above gastric content [4, 5].

In Single Layer Floating Tablets or Hydrodynamically Balanced System (HBS), Co_2 generating agents and the drug were mixed thoroughly within the matrix tablet to produce a formulation. And to remain buoyant in the stomach without effecting the gastric emptying rate for a prolonged period of time. The drug is released slowly at a desired rate [6,8]. When the drug is completely released the system is expelled out from the stomach which leads to an increased GRT and better control over fluctuation in plasma drug concentration [7,10].In Sublimation System, Camphor is used as a sublimation material. It is sublimated into the tablet matrix, forming pores in the matrix above the sublimation temperature. This system is useful to develop a porous floating matrix tablet using the sublimation method [11].

Beclomethasone dipropionate is the dipropionate ester of synthetic glucocorticoids possessing anti-inflammatory and immunomodulating properties. Medically it is used as steroid .It acts as a prodrug for the free form, beclomethasone (INN). Various available forms of it include inhaler (long term management of asthma), cream (dermatitis and psoriasis), pills (ulcerative colitis), and nasal spray (allergic rhinitis and nasal polyps) [12, 13]. Orally it is used to treat mild to moderate Crohn's disease of ileal or ileal right colonic localization Topical application of it is helpful to treat mild to moderate graft versus host disease**.** And it has a biological half life 2.8hrs**.**The aim of the present work is to prepare the gastro retentive floating tablets by using different concentrations of polymer using different methods by direct compression method and analyzing the release of drug from the tablets which is dependent on the concentration of polymer HPMC K4M. The main aim of present work is to prepare and evaluate highly porous gastro retentive floating tablets of Beclomethasone dipropionate [14, 15]

MATERIALS AND METHODS

Materials

Beclomethasone dipropionate is a gift sample from Halmak Pharmaceuticals Pvt.LTD, Ameerpet, Hyderabad, Hydroxy Propyl Methyl Cellulose K4M from Research Lab fine chem. industries, Mumbai, Micro Crystalline Cellulose from NR Chem., Bombay, Sodium bicarbonate from S.D Fine Chem. LTD, Mumbai, Magnesium stearate from KEMPHASOL-Bombay, Lactose from Qualikems Fine Chem. Industries, Mumbai, Talc from NICE Chemicals Private LTD.

Methods

Preparation of gastro retentive floating tablets is done using three different methods for each method three different formulations were prepared at different ratios.

The methods include: Effervescent method, non effervescent method and Sublimation method.

Direct compression method is used for preparation of tablets in all the three methods and mixing of powder was carried out in a blender for 15min followed by addition of magnesium stearate, lactose, and talc further mixed for 5min [1, 2,16].

Effervescent method

In this method 10mg of BD, polymers such as HPMC K4M, MCC were used in three different

ratios, 15mg of lactose as glidant, 5mg of magnesium stearate as lubricant, sodium bicarbonate acts as effervescent agent and citric acid as preservative were taken for tablet preparation.

Non effervescent method

In this method 10mg of BD, polymers such as HPMC K4M, MCC were used in three different ratios, 15mg lactose as glidant, 5mg magnesium stearate as lubricant were taken for the preparation of tablets.200mg of tablet was prepared in effervescent and non effervescent method.

Sublimation method

To the formulation prepared by non effervescent method addition of 100mg camphor is done. Camphor acts as sublimation material and weighed 300mg for each tablet fed manually into the die of an instrumented single punch tableting machine and directly compressed to make one tablet. Sublimation method formulated tablets were sublimated at 60^0C in hot air oven and the tablets weight were measured at regular intervals of time, camphor should be sublimated within 24 hrs completely.[1,2]

Table.1 formulation of beclomethasone dipropionate floating tablets using effervescent method

Ingredients	F1 (1:5)	F2 (1:6)	F3 (1:7)
BD	10	10	10
HPMC(K4M)	50	60	70
MCC	15	25	35
NAHCO3	90	80	70
citric acid	15	15	15
Mg Stearate	5	5	5
Talc	10	10	10
Lactose	15	15	15

Table.2 formulation of beclomethasone dipropionate floating tablets using non effervescent method

Ingredients	F4 (1:5)	F5 (1:6)	F6 (1:7)
BD	10	10	10
HPMC(K4M)	50	60	70
MCC	110	100	90
mg stearate	5	5	5
Talc	10	10	10
Lactose	15	15	15

Table.3 formulation of beclomethasone dipropionate floating tablets using sublimation method

Ingredients	F7 (1:5)	F8(1:6)	F9(1:7)
BD	10	10	10
HPMC(K4M)	50	60	70
MCC	110	100	90
mg stearate	5	5	5
Talc	10	10	10
Lactose	15	15	15
Camphor	100	100	100

EVALUATION TESTS

Floating ability

The floating ability of single tablet was determined with 500 ml pre warmed 0.1 N HCl solution and shaken at 70 rpm, 37 ± 0.2°C for 24 h, using a shaker apparatus.

Floating properties of tablets

In a 100 ml glass beaker 0.1 N HCl is taken to that solution tablets were added.

Floating Lag Time: The time required for a tablet to float on the surface of the medium is floating lag time.

Floating Duration Time: The duration of time where tablet remained floating on the medium surface was determined as floating duration time.

Precompression parameters

Precompression parameters were studied before punching a tablet.

Bulk density: It is the ratio of the known mass of the powder sample which is untapped and its volume including interparticulate void volume.

$$\text{Bulk density} = \frac{\text{Mass of the powder}}{\text{Bulk voume of the powder}}$$

It is measured in gm/ml.

Tapped density: It is the ratio of the mass of the powder to the volume occupied by the powder after a fixed number of taps.

$$\text{Tapped density} = \frac{\text{Mass of the powder}}{\text{Tapped volume of the powder}}$$

It is measured in gm/ml.

Compressibility index

$$\text{carrs index\%} = \frac{\text{tapped density} - \text{Bulk density}}{\text{Tapped density}} \text{ x } 100$$

Hausner's Ratio

$$\text{Hausner Ratio} = \frac{\text{tapped density}}{\text{bulk density}} \text{ x } 100$$

Angle of Repose: It is the minimum angle at which any piled up bulky or loose material stands without falling down. The angle of repose ranges from 0^0 to 90^o.

The tangent angle of repose (θ) was calculated by an equation[1,2,16]

$\tan \theta = h/r$

Angle of repose (θ) = tan-1(h/r)
Where,
h=height of the pile, r=radius of the pile, θ=angle of repose.

Drug-excipient (DE) interactions

FTIR: This is used to study the physical and chemical interactions between the drug and excipients used in the dosage form.

Differential Scanning Calorimeter (DSC): It determines the temperature and heat flow and also material transitions as a function of time and temperature.

Post compression parameters

Weight variation: Variations in weight were tested in randomly selected 20 different tablets from every batch. Digital electronic balance (Citizon CG203, India) is used for measuring weight variations. Then individual tablets were weighed and compared with an average weight. Weight values were reported in mg. Mean and SD were calculated.

Hardness (or) tablet crushing strength: The resistance of the tablets to capping, abrasion or breakage during storage, transportation and handling before usage depends on its hardness. Tablet hardness is the amount required to crush or fracture a tablet kept on its edge. Monsanto type (Make: Singhala) hardness tester is used for testing hardness. This instrument measures the crushing strengths.

It is measured in kg/cm^2.

Thickness: The diameter and thickness of the tablets were measured for 20 tablets from each formulation. Digital Vernier caliper is used for this study and it gives accurate measurements and information about variation between tablets. It is measured in mm.

Friability (F): Friability of the tablet is determined using Roche friabilator.Preweighed 20 tablets were subjected to the combined effect of abrasion and shock in a Friabilator containing a plastic chamber revolving at 25 rpm up to 100 revolutions. Remove the dust using soft muslin cloth, and then tablets were re-weighed and friability percentage was calculated using the following formula

$$\%\text{Friability} = \frac{\text{Tablet weight before friabilty} - \text{Tablet weight after friability}}{\text{Tablet weight before friabilty}} \text{ x } 100$$

Swelling Index: Individual weights of the floating tablets were taken (W0) and separately placed in a glass beaker which contains 200 ml of 0.1 N HCl or 50ml of water and incubated at 37°C ± 1°C. At regular 1-hr time intervals until 24 hrs, they were removed from beaker, and by using a tissue paper excess liquid on the surface was removed carefully. The swollen floating tablets were then re-weighed (Wt).The percentage of swelling index is given as

%Swelling index= $W_{wet} - W_{dry} / W_{dry}$ x 100

Invitro **Dissolution Study:** The release of beclomethasone dipropionate from the floating tablets was determined by using USP II paddle type dissolution test apparatus. This test is performed using 900 ml of 0.1 N HCl solution at 37 ± 0.5 °C and the paddles were rotated at 50 rpm. At regular time intervals, 5ml aliquot is withdrawn from the dissolution medium and it is replaced with fresh medium to keep the constant volume. The samples taken in aliquots were filtered and diluted with suitable concentrations of 0.1 N HCl. The absorbance was measured at 239 nm.

Kinetic Analysis of Dissolution Data

Zero order kinetics

$f_t = k_o t$

Where $f_t = 1 – (W_t / W_0)$ and ft represents the fraction of drug dissolved in time t .

First order kinetics

$\text{Log } Q_t = \text{Log } Q_0 + K_1 t/2.303$

Where Q_t is the amount of drug released at time t

Korsmeyer Peppa's model

$Q_t/Q\alpha = K_k t_n$

Where K_k is a constant and n is the release exponent that indicates drug release mechanism it is shown in Table no.4

Table.4 various mechanisms of drug transport

Release exponent(n)	Drug transport mechanism	Rate,the function of time
0.5	Fickian diffusion	$t^{-0.5}$
0.5<n<1.0	Anomalous transport	t^{n-1}
1.0	Case-II transport	Zero order release
Higher than 1.0	Super Case-II transport	t^{n-1}

Higuchi Model

$Q_t = K_H \, t \, 1/2$

A linear relationship between the square root of time versus the concentration implies that the drug release follows Fickian diffusion mechanism[1,2,16].

RESULTS AND DISCUSSION

Table.5 calibration curve of beclomethasone dipropionate

Concentration µg/ml	Absorbance
10	0.013
20	0.024
30	0.035
40	0.046
50	0.056
60	0.065
70	0.077
80	0.087
90	0.099

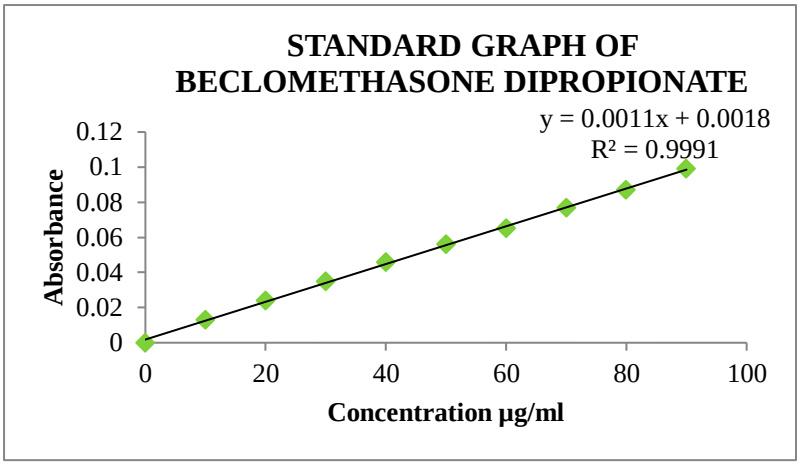

Figure.1 Standard calibration curve of pure beclomethasone dipropionate

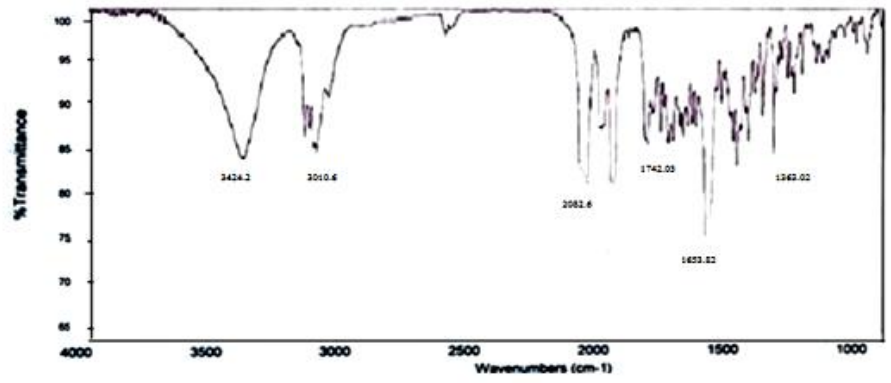

Figure.2 FTIR spectra of pure beclomethasone dipropionate

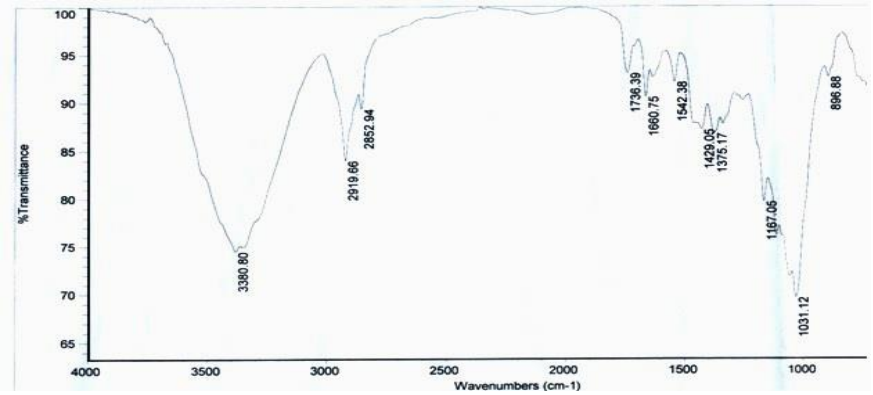

Figure.3 FTIR spectra of beclomethasone dipropionate gastro retentive floating tablets

Table.6 Interpretation of beclomethasone dipropionate and optimized formulation

Frequency of pure drug	Frequency of optimized drug	Frequency ranges	Functional group
3271	3380	3650-3200	Alcohol O-H
1730	1736	1735-1750	Aldehyde C=O
1654	1660	1650-1690	Amide C=O

It ensures that there is compatibility between pure drug and the optimized formulation

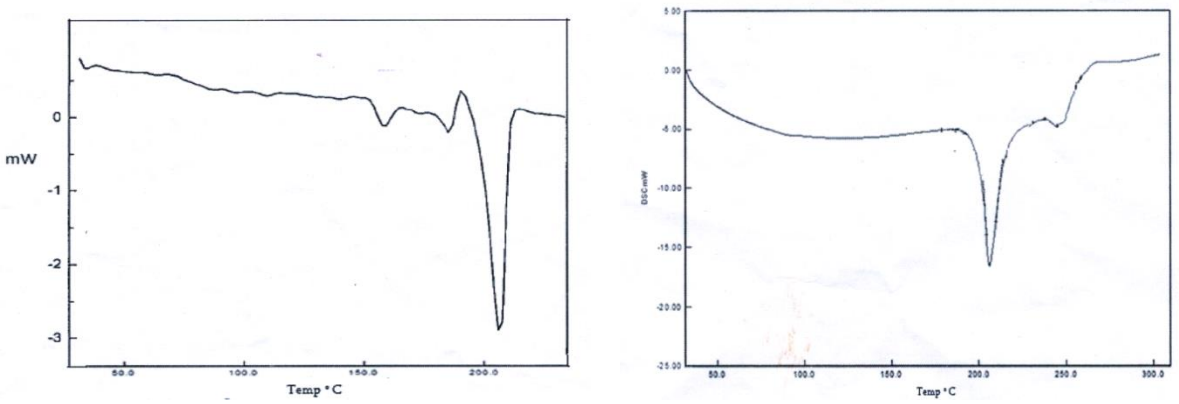

Figure.4 DSCof pure beclomethasone dipropionate

Figure.5 DSC of beclomethasone dipropionate floating tablets

Selected formulations of Gastro retentive floating tablets of Beclomethasone dipropionate were characterized for DSC. The pure Beclomethasone dipropionate showed a sharp endothermic peak at 213^{o} C. Similar endothermic peaks were observed at similar temperature in the prepared Gastro retentive floating tablets at 208^{o} C. The above results confirm that there was no drug excipient interaction.

Table.7 Flow Properties of Beclomethasone Dipropionate GR Floating tablets (F1 to F9)

Formulation	Angle of repose (θ^{o}) ± SD	Bulk density (g/ml)±SD	Tapped density (g/ml) ± SD	Carr's index (%) ± SD	Hausner's ratio Index ± SD
F1	24.15±0.06	0.51±0.03	0.62±0.05	17.74±0.07	1.21±0.06
F2	25.08±0.03	0.47±0.07	0.59±0.04	20.33±0.02	1.25±0.03
F3	23.28±0.12	0.48±0.05	0.59±0.06	18.64±0.02	1.22±0.03
F4	27.21±0.09	0.54±0.02	0.65±0.07	16.92±0.06	1.20±0.07
F5	28.25±0.02	0.52±0.03	0.63±0.03	17.46±0.05	1.21±0.05
F6	29.35±0.03	0.48±0.05	0.60±0.05	20.00±0.01	1.25±0.04
F7	26.75±0.05	0.47±0.04	0.56±0.06	16.07±0.06	1.18±0.08
F8	24.38±0.06	0.53±0.07	0.62±0.04	14.51±0.05	1.16±0.03
F9	25.02±0.08	0.50±0.02	0.58±0.03	13.79±0.08	1.15±0.05

Precompression parameters of beclomethasone dipropionate were determined and the results of them were shown in table. It ensures good flow property of powders. The results have shown acceptable range of flow properties.

Table.8 Physical Characterization of GR Floating tablets of B D (F1 to F9)

Formulation	Hardness (kg/cm^2)	Thickness (mm)	Diameter (mm)	Avg wt variation (mg)	Friability	Floating Lag time(Sec)	Floating Duration (hrs)
	4.5	5±0.13	9	195±0.13	0.45	118	>24
F1							
F2	5.3	4±0.74	9	200±0.16	0.48	158	>24
F3	5.0	5±0.76	9	200±0.16	0.44	155	>24
F4	4.6	5±0.12	9	205±0.25	0.51	105	>24
F5	4.7	4±0.13	9	200±0.17	0.47	200	>24
F6	4.7	4±0.79	9	197±0.12	0.45	250	>24
F7	4.3	6±0.02	9	200±0.17	0.46	0	>24
F8	4.0	7±0.02	9	200±0.10	0.53	0	>24
F9	4.0	7±0.01	10	200±0.25	0.46	0	>24

Table.9 swelling index data of Beclomethasone Dipropionate Floating tablets

Formulation	Swelling Index ±SD n=3
F1	5.03±0.12
F2	4.56±0.17
F3	4.12±0.26
F4	3.24±0.32
F5	4.01±0.27
F6	4.00±0.28
F7	4.60±0.23
F8	5.33±0.16
F9	6.20±0.18

The results of swelling index studies of GRF tablets of BD have shown in table. It has shown that by increasing the amount of polymer, swelling index was increased and it was determined for 6 hrs.

Invitro drug release studies

The dissolution studies were performed in USP type II apparatus using 0.1N HCL as a medium, maintained at a temperature of 37^0C for about 10 hours.

PERCENT CUMULATIVE DRUG RELEASE PROFILE

Table.10 In vitro Dissolution Data for Formulation F1 to F9 using HPMCK4M

	Cummulative %drugrelease				
Formulation	**1 hr**	**3hr**	**6hr**	**10hr**	**12hr**
F1	10.26±0.12	21.13±0.51	38.77±0.15	63.17±0.35	68.17±0.73
F2	10.98±0.26	24.47±0.35	40.50±0.24	65.61±0.56	68.72±0.59
F3	11.79±0.38	23.75±0.42	45.46±0.32	64.82±0.71	69.62±0.92
F4	13.5±0.56	25.35±0.85	48.00±0.62	69.04±0.46	70.72±0.49
F5	12.6±0.84	24.64±0.75	46.35±0.43	65.45±0.69	73.22±0.72
F6	14.4±0.65	26.40±0.49	52.15±0.61	71.50±0.73	75.77±0.37
F7	15.21±0.72	27.00±0.94	49.73±0.70	73.52±0.82	76.59±0.64
F8	16.02±0.59	28.72±0.45	51.57±0.86	75.44±0.19	79.12±0.53
F9	16.83±0.28	27.82±0.61	56.40±0.53	76.58±0.25	80.84±0.09

Invitro drug release study for all the nine formulations was carried out for 12hrs and tabulated shown in table. Formulation F9 met the desired drug release profile in 12 hr therefore, considered the best formulation among all the formulations.

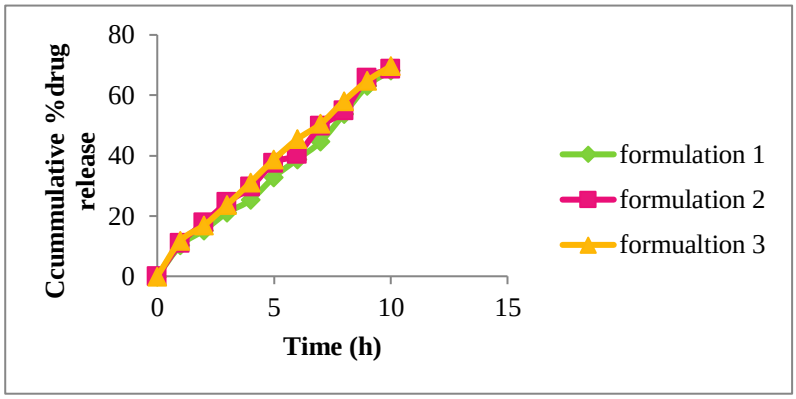

Figure.6 In vitro release of Beclomethasone dipropionate by using effervescent method

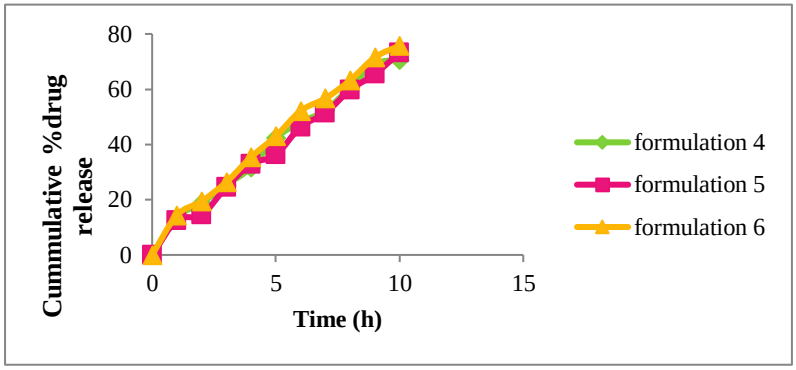

Figure.7 In vitro release of Beclomethasone dipropionate by using Non effervescent method

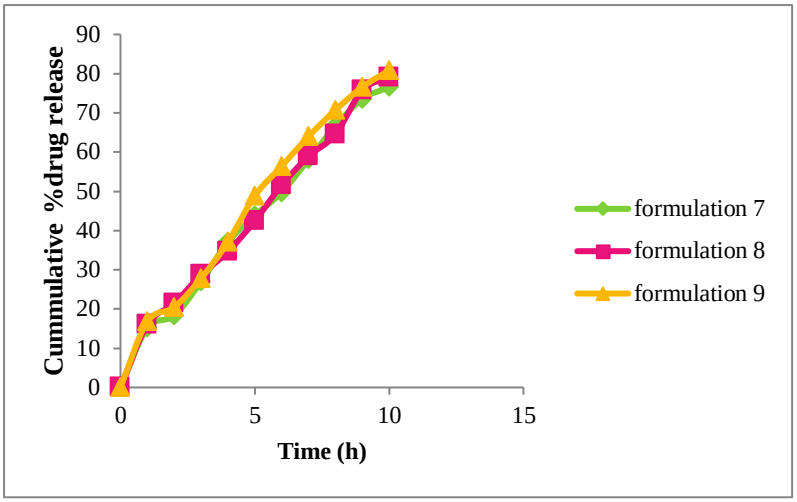

Figure.8 In vitro release of Beclomethasone dipropionate by using Sublimation method

Table. 12 Kinetic analysis of dissolution data for formulation 9

ZERO ORDER		**HIGUCHI MODEL**		**PEPPA MODEL**		**FIRST ORDER**	
Time (Hrs)	Cumulative % Drug Release	Sq.Root of Time	Cumulative% Drug Release	Log Time	Log % of Drug Release	Time (Hrs)	Log of %drug remaining
0	0	0	0	0	0	0	0
1	16.83	1.00	16.83	0.00	1.22	1	1.91
2	20.44	1.41	20.44	0.30	1.33	2	1.90
3	27.82	1.73	27.82	0.48	1.44	3	1.85
4	37.04	2.00	37.04	0.60	1.56	4	1.79
5	48.87	2.24	48.87	0.70	1.68	5	1.70
6	56.40	2.45	56.40	0.78	1.75	6	1.63
7	64.00	2.65	64.00	0.85	1.80	7	1.55
8	70.70	2.83	70.70	0.90	1.84	8	1.46
9	76.58	3.00	76.58	0.95	1.88	9	1.36
10	80.84	3.16	80.84	1.00	1.90	10	1.28

Table.13 Correlation coefficient (R2) and release exponent (n) values for different kinetic models of Formulation F9

Model name	**R^2 value**	**Slope**
Zero order model	0.986	8.051
First order model	0.980	-0.073

Higuchi's model	0.975	32.67
Korsmeyer-peppas model	0.972	0.788

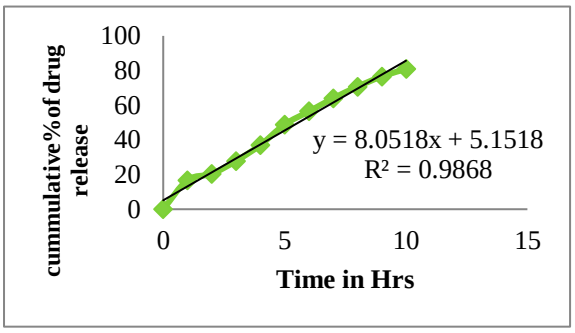

Figure.9 Zero order kinetics of Formulation F9

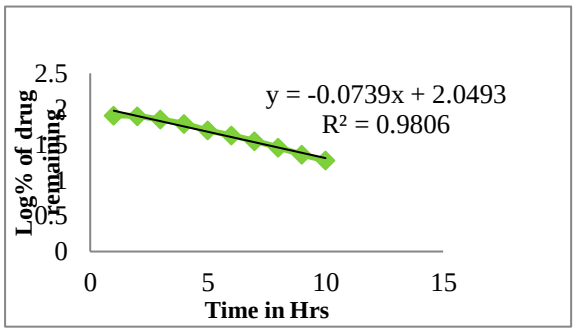

Figure.10 First order kinetics of Formulation F9

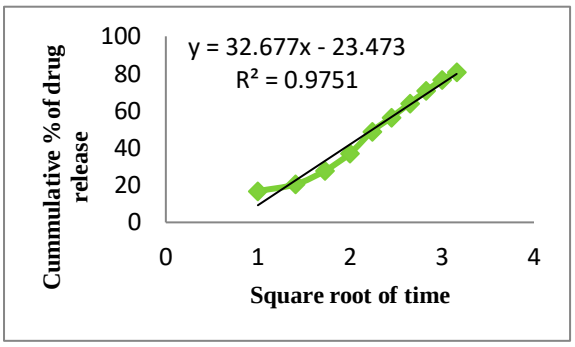

Figure.11 Higuchi model of Formulation F9

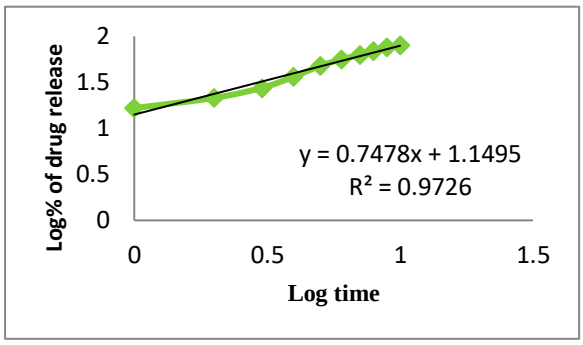

Figure.12 Peppa's model of Formulation F9

Correlation coefficient (R2) and release exponent (n) values for different kinetic models were shown in above table. The n value from the Korsmeyer-peppas model for GRF tablets of BD tablets is 0.747, which is less than 0.89, which shows Anomalous transport of diffusion.

Conclusion

The formulations of Gastro retentive floating tablets of Beclomethasone Dipropionate from F1 to F9 with HPMC K4M polymer in different ratios using three different methods such as effervescent, non effervescent method, and sublimation method were prepared. The formulation F9 prepared has shown 73% of drug release in 12hrs which uses camphor as sublimation material. These tablets has no lag time and floated for over >24hrs.The tablets prepared from F9 formulation retained the drug release up to desired time period due to the presence of pores and polymers such as HPMCK4M and MCC as both the polymers are swellable substances. The drug release kinetics, FTIR and DSC studies of F9 formulation indicated that in the tablets the drug was stable.

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FORMULATION AND INVITRO EVALUATION OF SUSTAINED RELEASE SELEGILINE MINI TABLETS

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ABSTRACT

The aim of the present study is to formulate and evaluate Selegiline sustained release Mini tablets. In the present study, an attempt was made to prepare mini-tablets of Selegiline by direct compression method using three polymers HPMCK4M, HPMCK15M, HPMCK100M with Magnesium Stearate, micro crystalline cellulose as co-excipients. The mini tablets of Selegiline was prepared by direct compression method. This method is suitable for high dose drugs which have poor flowability, binder which enhance the compressibility, cohesive property and the dissolution rate of insoluble drug can be enhanced. It is also suitable for bulky and dust producing powders. The Prepared matrix tablets were evaluated for pre compression and post compression Parameters like Bulk density, Carr's index, Angle of Repose, hardness test, friability test, weight variation test, drug content, uniformity test *in-vitro* drug release study. Increasing the amount of HPMC K15M ,the tablets resulted in the increase in the drug release rate and linearization of the drug release curve and the release of drug from the formulations was governed by non-Ficki and effusion mechanism. During dissolution process, it was observed that increase in the amount of polymer in the tablets resulted in a reduction in the drug release rate. Direct compression method is the best method for the formulation of mini tablets. HPMCK15 was found to be the best polymer among all, with 15% concentration yielding the best results for the core Mini tablets. Among all the formulations, F8 formulation shown maximum drug release hence it was considered as optimized formulation.

Key Words: Selegiline, mini tablets, Sustained Release drug delivery, HPMCK4M, HPMCK15M, HPMCK100M.

Dr.T.Mangilal,P.Aparna,J.Naveen, Formulation and *invitro* Evaluation of sustained release selegiline mini tablets, Asian Journal of Pharmaceutical Sciences,2018, 9 (5), 34-39 .

INTRODUCTION

Tablets are one of the most stable and commonly administered oral dosage forms. Since the later part of nineteen-century, tablets have been wide spread and their popularity continued. Tablets remain popular as dosage form because of the advantages afforded to both the pharmaceutical manufacturers and patients. These includes:Simplicity and economy of preparation, Stable and convenient in packing, Ease of transporting and dispensing, Accuracy of single dosage regimen, Compactness and portability and Blandness of taste and ease of administration.

The goal in designing sustained or controlled drug delivery systems[1] is to reduce frequency of dosing or to increase the effectiveness of the drug by localization at the site of action, reducing the dose required, providing uniform drug delivery ".If one were to imagine the ideal drug delivery system, two prerequisites would be required. First, it would be a single dose for duration of treatment, whether it is for days of weeks, as with infection, or for lifetime of the patient, as in hypertension or diabetes. Second, it should deliver the drug directly to the site of action, thereby minimizing or eliminating side effects. This may necessitate delivery to specific receptors or to localization to cells or to specific areas of the body. Oral ingestion has been the most convenient and commonly employed route of drug delivery. Indeed, for sustained release systems, oral route of administration has received most of the attention with respect to research on physiological and drug constraints as well as design and testing of products. This is because of the fact that there is more feasibility in dosage form design for oral route than for parenteral or any other route. The design of oral sustained release delivery systems is subject to several intercalated variables of considerable importance.

Sustained release drug delivery systems[2]

During the past few years, conventional dosage forms of drugs are rapidly being replaced by the new and the novel drug delivery systems .Amongst, these the controlled release/sustained release dosage forms have become extremely popular in modern therapeutics. The basic rationale for sustained release drug delivery is to alter the pharmacokinetics and pharmacodynamics of drugs by using novel drug delivery systems or by modifying the molecular structure or physiological parameters in here in as elected route of administration. It is desirable that the duration of drug action becomes more a design property of a rate controlled dosage form and less or not at all a property of the drug molecule's inherent kinetic properties.

Thus, optimal design of a sustained/controlled release system necessitates a thorough understanding of the pharmacokinetics and pharmacodynamics of the drug. When the drug is administered in a conventional dosage form, it results in a fluctuation of drug concentration at the site of action (peak and valley pattern)and therefore in systemic circulation and tissue compartment.

Advantages

1. Decreased local and systemic side effects.
2. Reduced gastrointestinal irritation.
3. Better drug utilization reduction in total amount of drug used.
4. Improved efficiency in treatment, optimized therapy, more uniform blood concentration.
5. Reduction in fluctuation in drug level and hence more uniform pharmacological response, cure of control of condition more promptly, less reduction in drug activity with chronic

use.

Disadvantages

1. Decreased systemic availability in comparison to immediate release conventional dosage forms, which may be due to incomplete release, increased first-pass metabolism, increased in stability, insufficient residence time complete release, site specific absorption, pH dependent stability, etc.
2. Poor *in vitro – in vivo* correlation.
3. Retrieval of drug is difficult in case of toxicity, poisoning or hypersensitivity reactions.
4. Reduced potential for dose adjustment of drugs normally administered in varying strengths.

Mini Tablets[12]

Mini-tablets are small tablets with a diameter typically equal to or less than 4mm that are filled into a capsule, or occasionally, further compressed into larger tablets. Several mini-tablets can be filled into hard capsules that, after disintegration, release these subunits as multiple dosage forms.

Advantages

Convenient advantages for patient

- Mini-tablets pack large quantity of active ingredient in a form that can be easily swallowed and thus ideal for administrating to paediatrics and geriatrics.
- They pass quickly and uniformly through the stomach regardless of the meal. After disintegrating, the individual subunit particles pass rapidly through the GI tract. If these subunits have diameters of less than 5mm, they are able to leave the stomach continuously, even if the pylorus is closed. These results in lower intra and inter individual variability in plasma levels and bioavailability.
- It is possible to incorporate many different mini-tablets, each one formulated individually and programmed to release drug at different sites within the gastrointestinal track, into one capsule. These combinations may include immediate release, delayed release, and/or controlled release Mini-tablets.
- It is also possible to incorporate Mini-tablets of different drugs to treat concurrent disease or combinations of drugs to improve overall therapeutic outcome, while delivering distinct release rates of each according to disease requirements.

Convenient advantages during manufacturing[13]

- Manufacturing different doses in only one tablet-forming step. Then the capsules are filled according to dose.
- Good alternative to pellets, and easier to manufacture.

- On demand, with retarding coating. This opens the opportunity to combine tablets with a different coating into one capsule such that chemical stability, physical appearance and patient compliance can be improved.
- The easy way to generate complex release profiles, i.e. initial and maintenance dose in one capsule lowers the risk for "dose dumping".
- Several chemically incompatible drugs pressed into Mini-tablets, coating and combined in one single capsule.

MATERIALS

Selegiline is a gift sample from Drugs India, Pvt Ltd, Hyderabad, Telangana, India. HPMCK4M, HPMCK15M, HPMCK100M, micro crystalline cellulose, Magnesium Stearate, Talc from Drugs India, Pvt Ltd, Hyderabad, Telangana, India. Otheringredients were of analytical grade and purchased from local markets.

METHODS

Pre formulation studies [14-18]

A. Organoleptic Properties: The color, odor and taste of the model drug were evaluated and tabulated using descriptive terminology.

B. Melting Point: Melting point of the drug was determined by capillary tube method.

C. Solubility Study: Solubility of the drug was determined by Shake Flask Method. According to this method compound is added to various solvents and shaken for 24hrs in Orbital Shaker. The saturation is confirmed by observation of undissolved material.

D. Loss On Drying: The accurately weighed 1gm of sample was transferred in glass-stoppered, shallow weighing bottle and accurately weighed the bottle. The bottle was transferred in oven and substance was dried at 105°C for 3 hours. The bottle was removed from oven and reweighed; loss on drying was calculated by following equation,

$$\text{LOD} = \frac{\text{Initial weight of substance} - \text{Final weight of substance}}{\text{Initial weight of substance}} \times 100$$

Analytical methods

A. λ max Determination: The absorption maximum of the standard solution was scanned between 200-400 nm regions on Shimadzu-1700 UV spectrophotometer. The absorption maximum obtained with the substance being examined corresponds in position and relative intensity to those in the reference spectrum.

B. Preparation of standard graph of Selegiline:

i. Preparation of 0.1N hydrochloric acid:0.1N HCl was prepared according to I.P. 1996. A quantity of 8.5 ml of HCl was diluted with fresh distilled water to produce 1000 ml.

ii. Preparation of stock solution of Selegiline: Accurately weighed 20 mg of Selegiline was dissolved in little quantity of distilled water and volume was adjusted to 100 ml with the same to prepare standard solution.

Procedure: From the stock solution, aliquots of 1, 2, 3, 4, 5, 6, 7, 8ml were transferred to 100 ml volumetric flasks and final volume was made to 100 ml with 0.01N HCl. Absorbance values of these solutions were measured against blank (0.01N HCl) at 205.5nm using Shimadzu-1700 UV spectrophotometer.

iii. Quantification of Drug: Accurately weighed 20 mg of Selegiline was dissolved in little quantity of distilled water and volume was adjusted to 100 ml with the same to prepare standard solution. From the above solution, aliquots of 5 ml were transferred to 100 ml volumetric flasks and final volume was made to 100 ml with 0.01N HCl. Absorbance values of these solutions were measured against blank (0.01N HCl) at 236nm using Shimadzu-1700 UV spectrophotometer.

Compatibility Testing Of Drug with Polymer: The proper design and formulation of a dosage form requires consideration of the physical, chemical and biological characteristics of all drug substances and excipients to be used in the fabricating the product. Each polymer used in the formulations was blended with the drug levels that are realistic with respect to the final dosage form. Each polymer was thoroughly blended with drug to increase drug- polymer molecular contacts to accelerate the reactions if possible.

Fourier transforms infra-red spectroscopy[19-20] :FTIR study was carried out to check compatibility of drug with polymers. Infrared spectrum of Selegiline was determined on fourier transform Infrared Spectrophotometer using KBr dispersion method. The base line correction was done using dried potassium bromide. Then the spectrum of dried mixture of drug and potassium bromide was run followed by drug with various polymers by using Parkin elmer-Pharmaspec-1 FTIR spectrophotometer.

Physical parameter studies [21-24]

i) Bulk Density: An accurately weighed powder blend from each formula was lightly shaken to break any agglomerates formed and it was introduced in to a measuring cylinder. The volume occupied by the powder was measured which gave bulk volume. The bulk densities of powder blends were determined using the following formula.

Bulk density = Total weight of powder / Total volume of powder

ii)Tapped bulk density: An accurately weighed powder blend from each formula was lightly shaken to break any agglomerates formed and it was introduced into a measuring cylinder. The

measuring cylinder was tapped until no further change in volume was noted which gave the tapped volume. The tapped bulk densities of powder blends were determined using the following formula.

TBD= Total weight of powder / Total volume of tapped powder.

iii)Carr's Compressibility Index: It is a simple index that can be determined on small quantities of powder. The compressibility indices of the formulation blends were determined using following formula.

Carr's Compressibility Index (%) = [(TBD-BD)/ TBD] x100

iv) Hausner's Ratio: Hausner's ratio was determined by following equation

$$Hausner's\ ratio = \frac{TBD}{BD}$$

A Hausner's ratio less than 1.25 indicates good flow while greater than 1.5 indicates poor flow.

v) Angle of repose: The angle of repose was determined by the funnel method. The accurately weighed powder was taken in a funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touched the apex of the heap of the powder. The powder was allowed to flow through the funnel freely onto the surface. The diameter of the powder cone was measured. The angle of repose was calculated using the following equation.

$$\tan(\theta) = h/r$$

Where 'h' and 'r' are the height and radius respectively of the powder cone

Compression of powder blends into tablets: After evaluation of powder blend, the sustained release tablets were prepared by Direct Compression granulation method using (6mm diameter, round flat faced punches) multiple punch tablet compression machine. Each tablet contained 20 mg of Selegiline; the batch size for each formulation was 10 tablets.

Table 1: Composition of Sustained release tablet formulation

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Selegiline	20mg	20 mg	20 mg	20 mg	20 mg	20 mg	20 mg	20 mg	20 mg
HPMCK4M	25 mg	50 mg	75 mg	-	-	-	-	-	-
HPMCK15M	-	-	-	25 mg	50 mg	75 mg	-	-	-
HPMCK100M	-	-	-	-	-	-	25 mg	50 mg	75 mg
MCC	110mg	115mg	90mg	110mg	115mg	90mg	110mg	115mg	90mg
Magnesium Stearate	05mg	05 mg	05mg	05mg	05 mg	05mg	05mg	05 mg	05mg
Talc	10mg	10mg	10mg	10mg	10mg	10mg	10mg	10mg	10mg
Total wt.	200 mg	200 mg	200mg	200 mg	200 mg	200 mg	200 mg	200 mg	200 mg

Evaluation of sustained release matrix tablets [25]

i)Appearance: The tablets were visually observed for capping, chipping, lamination and colour

ii) Dimension (Thickness and Diameter): The thickness and diameter of tablets were important for uniformity of tablet size. The thickness and diameter of the tablets was determined using a Vernier caliper.

iii) Tablet Hardness: For each formulation, the hardness of 6 tablets was determined using the Monsanto hardness tester. The tablet was held along its oblong axis in between the two jaws of the tester. At this point, reading should be zero kg/cm^2. Then constant force was applied by rotating the knob until the tablet fractured. The value at this point was noted in kg/cm^2.

iv) Friability: Five tablets were weighed collectively and placed in the chamber of the friabilator. In the friabilator, the tablets were exposed to rolling, resulting from the free fall of tablets within the chamber of the friabilator. After 100 rotations (i.e. In 4 minutes), the tablets were taken out from the friabilator and intact tablets were again weighed collectively. The difference in the weight is noted and expressed as a percentage. It should be nil or less than 0.1%Percent friability (% F) was calculated as follows,

% F = (Initial Wt. - Final Wt. / Initial Wt.) x 100

v) Drug Content Uniformity: Content uniformity was determined by accurately weighing 20 tablets and crushing them in mortar. Then an accurately weighed quantity of powder equivalent to 20 mg of drug was transferred to a 100 ml volumetric flask. Few ml of water was added and shaken for 15 min. Volume was made up to 100 ml with distilled water. The solution was filtered through Whatmann filter paper. 5 ml of the filtrate was diluted to 100 ml with 0.1N hydrochloric acid. Then absorbance of the resulting 10 μg/ml solution was recorded at 236nm. Content uniformity was calculated using formula –

% Purity = 10 C (Au / As)

vi) Weight Variation: 20 tablets of each formulation were weighed individually using an electronic balance, average weight was calculated and individual tablet weight was then compared with average value to find the deviation in weight.

Average Weight=Weight of 20 tablets/20

***In-vitro* dissolution studies[26-28]:** The *invitro* dissolution was carried out using USP type I dissolution apparatus Dissolution medium: 0.1N hydrochloric acid for first 2 hours,pH 6.8 phosphate buffer for next 8 hours,Temperature of dissolution medium: 37 ± 1^0 C.

The tablets were placed in the dissolution medium and the apparatus was run. At intervals of 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10 hours 5 ml aliquots were withdrawn and replacement was done each time with equal amounts of fresh dissolution medium maintained at same temperature. Each 5 ml

aliquot was filtered through Whatmann filter paper (No.41). 5 ml of sample was diluted to 10 ml 0.1N Hydrochloric acid for first 2 hours and then with pH 6.8 phosphate buffer for next 8 hours and absorbance of these solutions was measured at 228nm using a Shimadzu-1700 UV spectrophotometer. Drug concentrations in the sample were determined from standard calibration curve. The release data were calculated by using PCP disso V3 software.

Release kinetics [29-35]: To study the release kinetics of *in-vitro* drug release, data was applied to kinetic models such as Zero order, First order, Higuchi and Korsmeyer- Peppas.

Zero order

$$C = K_0 t$$

Where K_0 - is the zero-order rate constant expressed in units of concentration/time

t -is the time in hrs.

First order

$$LogC = LogC_0 - Kt / 2.303$$

Where C_0 - is the initial concentration of drug,

K - is the first order constant

t - is the time in hrs.

Higuchi

$$Qt = Kt^{1/2}$$

Where Q_t - is the amount of the release drug in time t,

K- is the kinetic constant and

t- is time in hrs

Korsmeyer Peppas

$$Mt/M\infty = Kt\ n$$

Where M_t - represents amount of the released drug at time t,

M_∞ is the overall amount of the drug (whole dose) released after 12 hrs

K- is the diffusional characteristic of drug/ polymer system constant

n- is a diffusional exponent that characterizes the mechanism of release of drug.

RESULTS AND DISCUSSION

Preformulation parameters:

i) Organoleptic properties: Odourless, white and crystalline powder

ii) Melting point: Melting point values of Selegiline sample was found to be in range of 185^0C to 189^0C. The reported melting point range for Selegiline is 183.5^0C to 184^0C. Hence, experimental values are in good agreement with official values.

iii) Solubility study:

Table 2:The solubility of Selegiline in various solvents

Name of solvent	Inference
Distilled water	Freely soluble
Methanol	Very soluble
Iso propyl alcohol	Soluble
Acetonitrile	Sparingly soluble
Acetone	Slightly soluble
Chloroform	Slightly soluble
0.1N HCl	Soluble
0.01N HCl	Soluble
Phosphate buffer(pH6.8)	Soluble

Analytical methods:

λ max Determination: The absorption maximum for Selegiline was found to be 228nm.

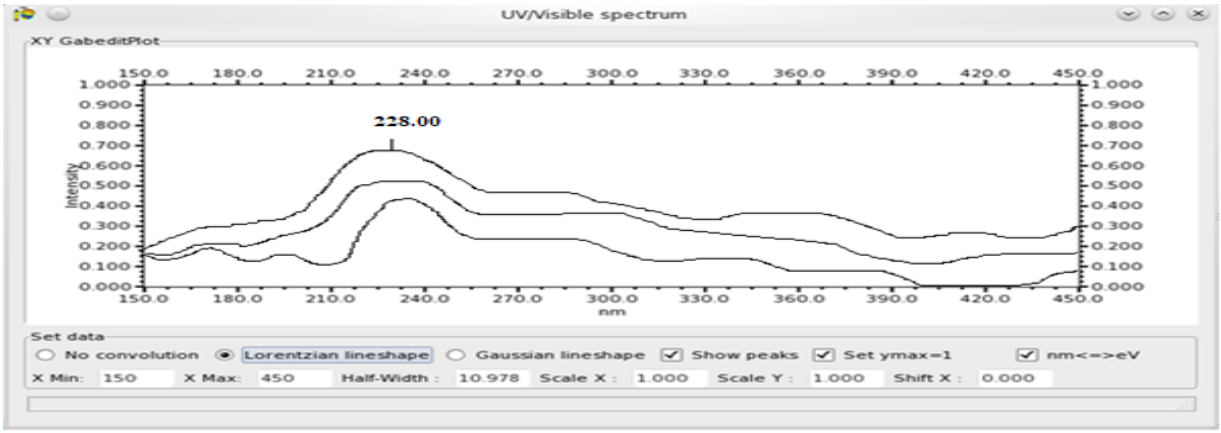

Figure 1 Spectrum of Selegiline

Preparation of standard graph of Selegiline

Preparation of standard graph of Selegiline in 0.1N HCl: UV absorption spectrum of Selegiline in 0.1N HCl shows λ max at 228nm. Absorbance's obtained for various concentrations of Selegiline in 0.1N HCl are given in table no 2. The graph of absorbance vs concentration for

Selegiline was found to be linear in the concentration range of 2-16 µg /ml. The drug obeys Beer-Lambert's law in the range of 2-16 µg /ml.

Preparation of standard graph of Selegiline in p^H 6.8 Phosphate buffer:UV absorption spectrum of Selegiline in p^H 6.8 Phosphate buffer shows λ max at 206nm. Absorbance's obtained for various concentrations of Selegiline in p^H 6.8 phosphate buffer are given in table no.3. The graph of absorbance vs concentration for Selegiline was found to be linear in the concentration range of 2-16 µg /ml. The drug obeys Beer- Lambert's law in the range of 2-16 µg /ml

Table 3: Data of concentration and absorbance for in Selegiline 0.1N HCl.

S.No	Concentration (µg/ml)	Absorbance
1	0	0.000
2	2	0.219
3	4	0.435
4	6	0.605
5	8	0.812
6	10	0.991
7	12	1.183
8	14	1.381
9	16	1.574

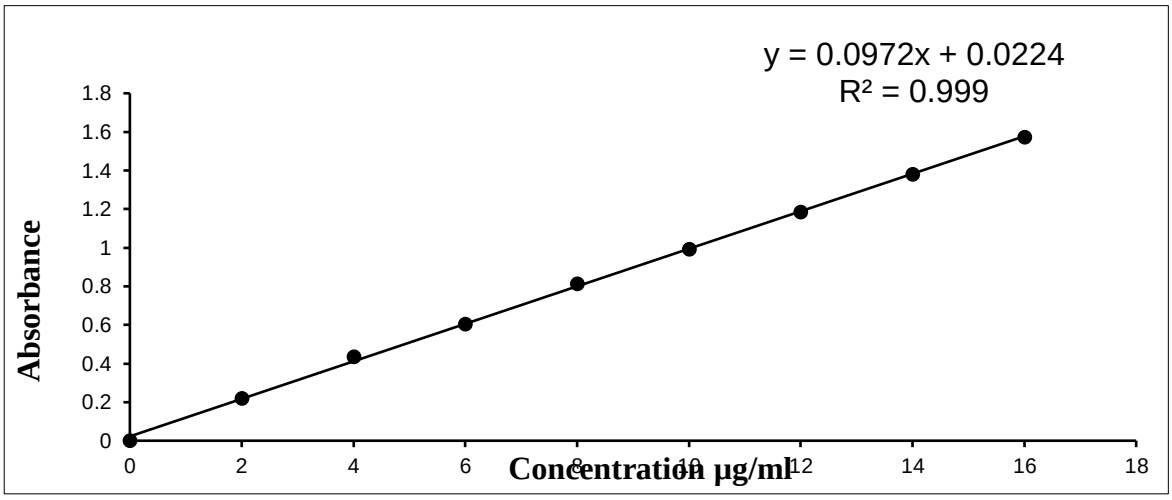

Figure 2 Standard graph of Selegiline 0.1N HCl

Table 4: Data of concentration and absorbance for Selegiline in pH6.8 phosphate buffer:

S. No	Con (µg/ml)	Absorbance
1	0	0.000

2	2	0.197
3	4	0.395
4	6	0.583
5	8	0.772
6	10	0.954
7	12	1.151
8	14	1.345
9	16	1.527

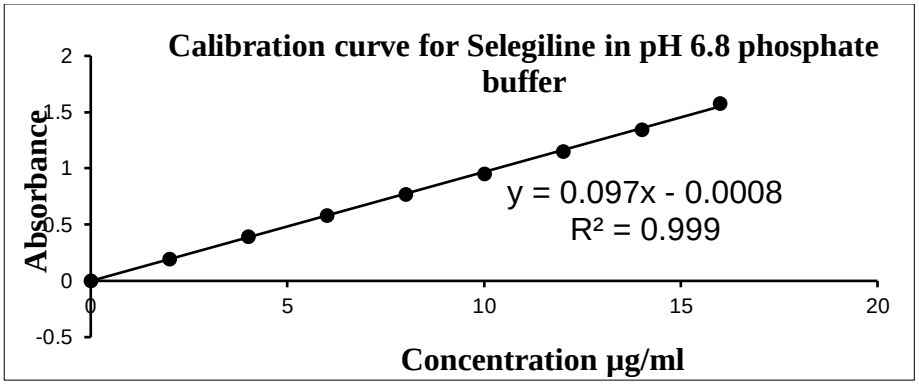

Figure 3: Standard graph of Selegiline pH 6.8 Phosphate buffer

Percentage purity of pure Drug: The percentage purity of drug was calculated by using calibration graph method (least square method).

Table 5: Percentage purity of pure drug

S. No	Percentage purity (%)	Avg. percentage purity (%)
1	100.60	99.69
2	99.08	
3	99.40	

The reported percentage purity for Selegiline is 98 to 102% (I.P. 1996).

Compatibility testing of drug with polymer

FTIR Spectroscopy: FTIR study was carried out to check compatibility of drug with polymers. Infrared spectrum of Selegiline was determined on Fourier transform infrared spectrophotometer using KBr dispersion method. The base line correction was done using dried potassium bromide. Then the spectrum of dried mixture of drug and potassium bromide was run followed by drug with various polymers by using Parkin elmer-Pharmaspec-1 FTIR spectrophotometer.

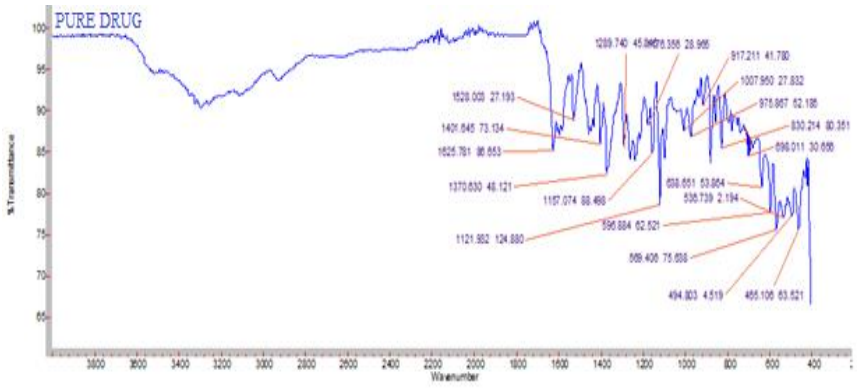

Figure 4:FTIR spectrum of pure drug

Figure 5: FTIR spectrum of optimized formulation

By observing the above FTIR spectrums it was observed that there is no significant change in the peaks observed in the pure drug spectrum to the peaks observed in the optimised formulation therefore it was concluded that there is no incompatability between the pure drug and the polymers used for the formulation

Characterization of powder blend: The powder blends were prepared by mixing of various ingredients mentioned in table and used for characterization of various flow properties of powder.

Bulk Density: The powder blends of formulations have the bulk density ranged between 0.774±0.0005 to 0.786±0.005 gm/ml.

Tapped bulk density : The powder blends of formulations have the tapped bulk density ranged between 0.867 to 0.898g/ml. These values indicate good packing characteristics and the powder was not bulky.

Carr's Compressibility Index: The carr's index for all the formulations was found to be below 12% indicating that the powders have a excellent compressibility.

Hausner's Ratio: The hausner ratio for all the formulations was found to be <1.25, indicating good flow properties.

Angle of repose: The flow properties of were analyzed by determining angle of repose which was found to be between 20.07 to 23.08, indicating 22.11excellent flow property.

Post Parameters

Appearance: The tablets were observed visually and did not show any defect such as capping, chipping and lamination.

Dimension (Thickness and Diameter): The diameter of the tablets of all formulationsF1-F9 were found to be 4.0±0.0 mm and thickness ranged between 2.10to 2.18.

Tablet Hardness: The hardness of tablets was found to be in the range of 4.5kg/cm^2 to 5 kg/cm^2. This indicates good tablet strength.

Table 6: Post Parameters of Selegiline Mini tablets

S.no	Formulation	Weight variation(mg)	Thickness (mm)	Hardness kg/cm^2	Friability
1	F1	0.568	2.5	5	0.26
2	F2	0.835	2.6	5	0.62
3	F3	0.545	2.7	5	0.78
4	F4	0.563	2.7	5	0.79
5	F5	0.927	2.1	4.5	0.41
6	F6	0.746	2.4	5	0.38
7	F7	0.835	2.5	5	0.78
8	F8	0.545	2.6	5	0.79
9	F9	0.563	2.7	4.5	0.41

Friability: Friability of all the formulations was found between 0.284±0.008 to 0.454±0.054%. This indicated good handling property of the prepared SR tablet.

Weight Variation: A tablet is designed to contain a specific amount of drug. When the average mass of the tablet is 200 mg the Pharmacopoeial limit for percentage deviation is ± 5 %. The percentage deviation from average tablet weight for all the tablet was found to be within the specified limits and hence all formulations complied with the test for weight variation according to the Pharmacopoeial specifications.

Drug content of Selegiline: The content of active ingredients in the formulation was found to be between 98.54±1.7 to 100.86±1.2 % w/w, which is within the specified limit as per IP 2007(i.e. 90-110% w/w).

***In-Vitro* Dissolution Studies**

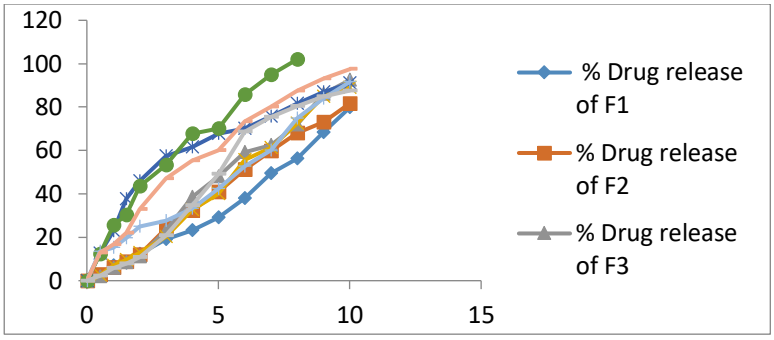

Figure 6 Dissolution graph of F1-F9 formulations

Table8 dissolution data comparision of optimized formulation F8 with marketed Selegiline
Formulation (selgin-5mg)

Hours	Dissolution medium	% Drug release of F8	% Drug release of marketed formulation-MF
0	0.1 N HCl	0	0
1		12.87	5.46
2		16.77	12.34
3		22.09	19.37
4		33.03	27.64
5		47.15	36.15
6		55.38	43.16
7	pH 6.8 phosphate buffer	80.27	69.48
8		87.44	77.84
9		93.24	85.46
10		97.47	91.44

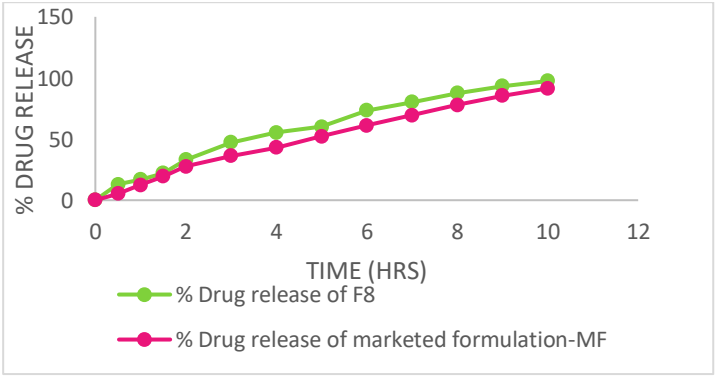

Figure 7 Dissolution graph dissolution data comparision of optimised formulation f8 with marketed selegiline formulation (selgin-5mg)

Various sustained release formulations were formulated with HPMC K4M, K15M, K100M, polymer alone; and microcrystalline cellulose was used as diluent. The drug release data of dissolution studies of formulation f8 containing HPMC K100M is shown concentration levels were found to be 97.47% respectively.

Kinetics Of *In-Vitro* Drug Release

Table 9 Different kinetic models for Selegiline Multi-Particulate Mini tablets

CUMULATIVE (%) RELEASE Q	TIME (T)	ROOT (T)	LOG(%) RELEASE	LOG (T)	LOG (%) REMAIN
0	0	0	0	0	2.000
12.87	0.5	0.707	1.110	-0.301	1.940
16.77	1	1.000	1.225	0.000	1.920
22.09	1.5	1.225	1.344	0.176	1.892
33.03	2	1.414	1.519	0.301	1.826
47.15	3	1.732	1.673	0.477	1.723
55.38	4	2.000	1.743	0.602	1.650
60.19	5	2.236	1.780	0.699	1.600
73.38	6	2.449	1.866	0.778	1.425
80.27	7	2.646	1.905	0.845	1.295
87.44	8	2.828	1.942	0.903	1.099
93.24	9	3.000	1.970	0.954	0.830
97.47	10	3.162	1.989	1.000	0.403

Zero order kinetics

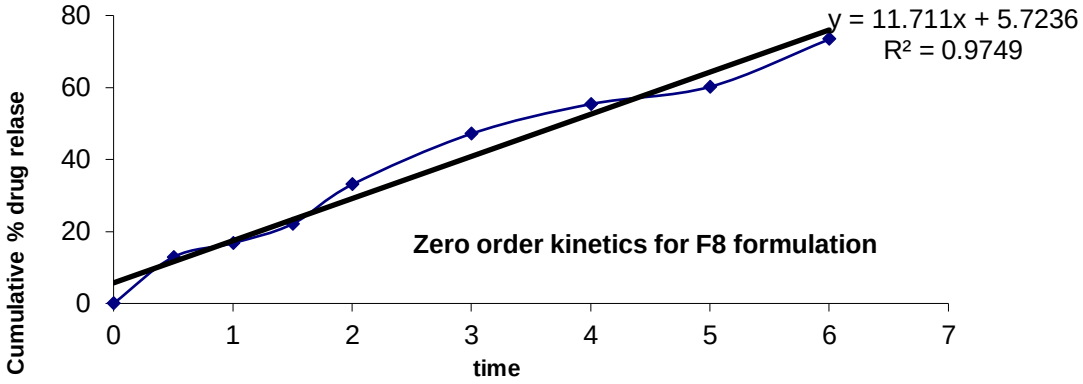

Figure 8 Zero order kinetics

Higuchi plot

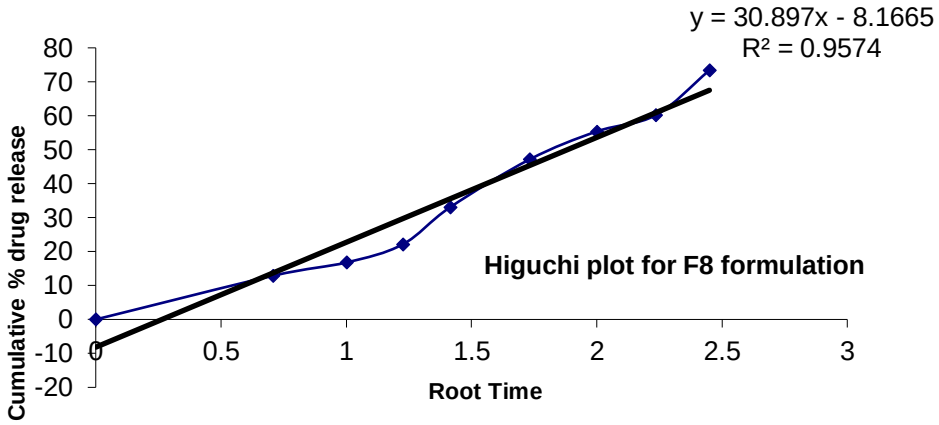

Figure 9 Higuchi plot

Peppas plot

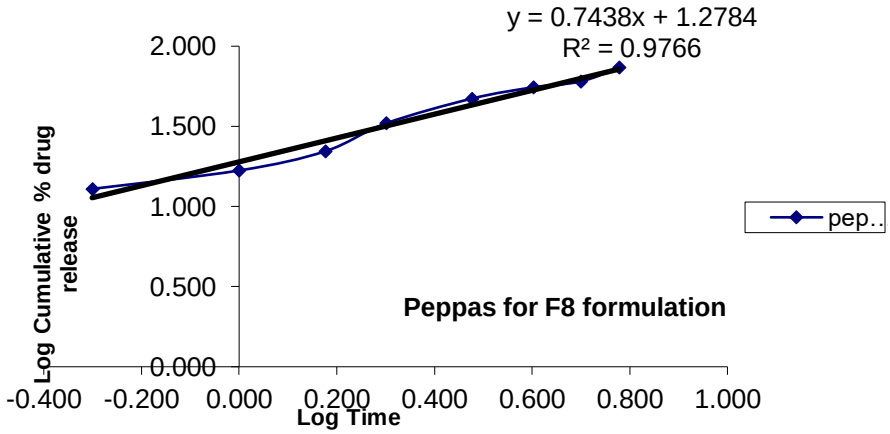

Figure 10 Peppas plot

First order kinetics

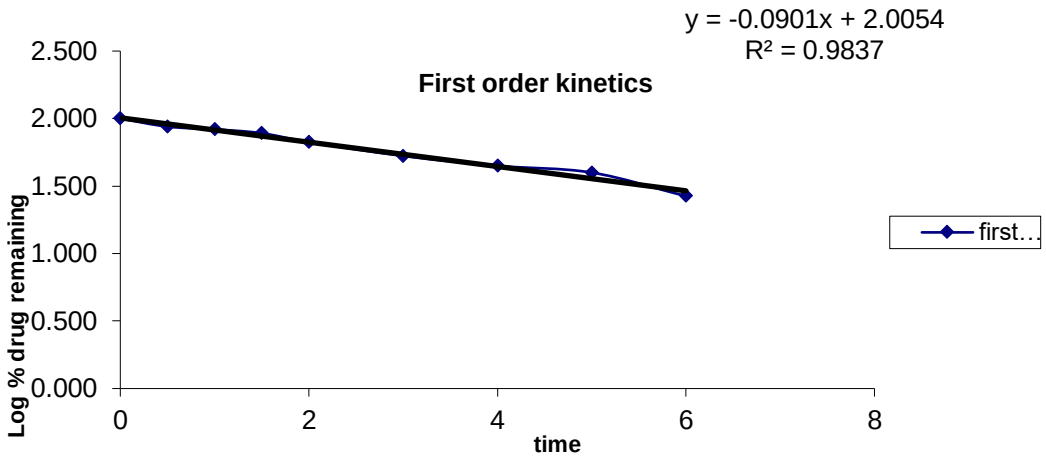

Figure 11 first order kinetics

CONCLUSION

In view of the findings in Results it can be suggested that formulations containing HPMC K100M can be employed successfully for the development of Mini tablets of Selegiline. All the formulations showed high process yield and drug loading capacity. Among the different batches, Formulation F8 was selected as the optimized formulation, after considering the *in vitro* drug release. Release kinetics studies showed that Sustained Release of Selegiline Mini Tablets were better fitted to first order and Higuchi models as indicated by higher r^2 values, which indicates the drug release was first order diffusion controlled. The n value of Korsmeyer Peppas equation indicates the release mechanism was non-Fickian. Based on the observations, it can be concluded that the formulated F8 tablets of Selegiline widely accepted and physiologically safe. Polymer was capable of exhibiting sustained release properties for a period of 10 hrs. They are thus may be reduce frequency of dosing, thereby minimizing the occurrence of side effects, improve bioavailability and increase the effectiveness of the drug.

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DEVELOPMENT AND CHARACTERIZATION OF COLON PH TRIGGERED MULTIPARTICULATE DRUG DELIVERY SYSTEM FOR ULCERATIVE COLITIS

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ABSTRACT

The purpose of the present study was to develop and characterize mesalamine microsphere to target colon for ulcerative colitis. Mesalamine is a drug with low solubility so in order to increase its solubility the solid dispersions of mesalamine were prepared by using kneading method with three different carriers, poly vinyl pyyrolidone (PVP K-30), poly ethylene glycol (PEG 4000) and β-cyclodextrin and evaluated for solubility determination. The solid dispersion (F15) drug: β-cyclodextrin (1:3) was selected for further formulation of microspheres due to its high solubility (4.268±0.031 mg/ml). The microspheres were prepared by using solvent evaporation method. The polymers used were Eudragit L 100 and Eudragit S 100 and glutaraldehyde is used as cross linking agent. The prepared formulations of microspheres were characterized for various parameters like particle size analysis, Scanning electron microscopy, micromeritic properties, percentage yield, drug content, entrapment efficiency, *in-vitro* release studies and stability studies. The micromeritic properties like angle of repose, Hausner's ratio and Carr's index showed good flow properties for all the formulations. The particle size of all formulations was in the range of 203±14 to 437±24 µm. The *in-vitro* studies revealed that the M6 formulation showed the drug release of 95.12% in acontrolled manner up to 14 hrs and the best fit model was Hixson and Crowell which shows that drug release is mainly by dissolution.

Shiva Kumar Yellanki, B Mamatha. Development and Characterization of Colon pH Trigerred Multiparticulate Drug Delivery System for Ulcerative Colitis. Inventi Rapid,2018,2,1-5

INTRODUCTION

Delivery of drugs to the colon, via Oral route is valuable in treating diseases of the colon (Ulcerative colitis, amoebiasis, crohn's disease, carcinoma and infections) where high local concentration of drug can be achieved with fewer side effects and the most critical challenge in one such approach is to protect the dosage form in the upper GIT and also to prevent unnecessary systemic absorption. [1] By targeting the drug to the colon, the maximum concentration of drug reaches and increases the residence time of drug in the colon. [2] The various approaches in targeting the drug to the colon include pH dependent system, time dependent system, microbially triggered approach. [3] The newly developed approaches include pressure controlled DDS, osmotic controlled DDS, CODES™ system, Ticking capsule, Enterion capsule etc.

Many techniques are available for enhancing the dissolution characteristics of slightly water soluble drugs such as Micronization, formation of solvates, complexes, adsorbates, solid dispersion. Solid dispersion refers to a group of solid products consisting of atleast two different components generally hydrophilic carrier and a hydrophobic drug. [4] The different methods of preparation of solid dispersions are Solvent evaporation method, fusion/ melting method, Kneading method, Physical mixture method, super critical fluid technology. [5]

Microencapsulation technique has been mostly used for the lipophilic drugs since hydrophilic drugs show low loading efficiency. [6] Microspheres can be defined as solid approximately spherical particles, ranging in size from 1 to 1000 μm. [7]

The aim of the present study was to formulate and evaluate the multiparticulate DDS of Mesalamine. Firstly the solid dispersion of mesalamine was prepared by using PVP K-30, PEG 4000, β-Cyclodextrin to enhance the solubility. The solubility was determined for all the formulations. The formulations with highest solubility were used to prepare microspheres by using Eudragit L 100 and Eudragit S 100 polymers and were characterized for various parameters.

MATERIALS AND METHODS

The drug Mesalamine, β-cyclodextrin and Glutaraldehyde was purchased from ALM Laboratories, Warangal. Eudragit L 100 and Eudragit S 100 were obtained as a gift sample from Evonik, Degussa, Mumbai. PEG 4000 was gifted by Tablets India, Chennai. PVP K-30 from Priyadarshini College of Pharmaceutical Sciences, Ghatkesar. All solvents used were of analytical grade.

Preparation of Solid Dispersion

Mesalamine and the various water soluble carriers (PVP K-30, PEG4000 and β Cyclodextrin) were weighed in different ratio and transferred to mortar were taken and mixed for 5 min and then kneaded for 45 min using hot water. To maintain paste like consistency sufficient hot water was added. The paste was then dried in hot air oven at 45°C for 24 hours. The dried products were crushed, pulverized and passed through sieve No. 60. The prepared dispersions were stored in glass vials and the solubility of the solid dispersions was determined. The ratios of solid dispersion were shown in Table 1.

Table 1: Formulation Table of Mesalamine Solid Dispersion and their Solubilities

Formulation Code	Drug (gm)	PVP K-30 (gm)	PEG 4000 (gm)	B-Cyclodextrin (gm)	Solubility Determination mg/ml (mean±SD)
F1	0.5	0.25	-	-	1.247±0.025
F2	0.5	0.50	-	-	1.519±0.011
F3	0.5	0.75	-	-	1.873±0.048
F4	0.5	1.0	-	-	1.917±0.023
F5	0.5	1.5	-	-	2.015±0.036
F6	0.5	-	0.25	-	2.498 ±0.063
F7	0.5	-	0.50	-	2.663±0.091
F8	0.5	-	0.75	-	2.859 ±0.054
F9	0.5	-	1.0	-	3.132±0.059
F10	0.5	-	1.5	-	3.296±0.071
F11	0.5	-	-	0.25	2.546±0.045
F12	0.5	-	-	0.50	2.736±0.028
F13	0.5	-	-	0.75	3.584±0.020
F14	0.5	-	-	1.0	3.898±0.016
F15	0.5	-	-	1.5	4.268±0.031

Table 2: Formulation of Mesalamine Microspheres

Ingredients	M1	M2	M3	M4	M5	M6	M7	M8	M9
Solid dispersion F15 (eq.wt to drug (mg))	250	250	250	250	250	250	250	250	250
Eudragit L 100 (mg)	250	375	500	-	-	-	125	187.5	250
Eudragit S 100 (mg)	-	-	-	250	375	500	125	187.5	250
Coconut oil (ml)	50	50	50	50	50	50	50	50	50
Ethanol (ml)	8	8	8	8	8	8	8	8	8
Acetone (ml)	2	2	2	2	2	2	2	2	2
1%v/v Span 80 (ml)	5	5	5	5	5	5	5	5	5
Glutaraldehyde (ml)	2	2	2	2	2	2	2	2	2

Determination of Solubility

Solubility study was carried out by using flask shaker method. Excess mesalamine and its different dispersions were introduced separately into the bottles with 25 ml capacity, each containing 25 ml of distilled water. All suspensions were protected from the light by wrapping the bottles with aluminum foil and shake for 24 hours at room temperature. The content of each bottle was then filtered through Whatmann filter paper. [10] The filtrate was then diluted with distilled water and assayed spectrophotometrically at 210 nm (ELICO double beam UV-visible Spectrophotometer). Solubility of each sample was determined in triplicate. The results are shown in Table 1. The F15 solid dispersion has shown a maximum solubility of 4.268±0.031 mg/ml, while the solubility of pure drug was found to be 0.996±0.037.

Formulation of Microspheres

The microspheres were prepared by using solvent evaporation technique. The F15 solid dispersion and the polymer were dissolved in 10ml of ethanol:acetone (4:1) using sonicator for 15 min. This solution was injected into 50 ml of coconut oil containing 1%v/v span-80 at a speed of 900 rpm with a lab stirrer. The temperature of the system is maintained at 30-40°C using Heating mantle. After 15 min, 2 ml of glutaraldehyde was added to the above solution, as a cross linking agent. After 3 hours the microspheres were filtered using Whatmann filter paper, washed with n-hexane to remove the oil and air dried at room temperature for 24 hrs.

Characterization of Mesalamine Microspheres

1. Particle Size Analysis

Particle size of the microspheres was evaluated using optical microscopy method. Approximately 100 microspheres were counted for particle size determination using a calibrated optical microscope. The experiment was performed in triplicate. [11]

2. Shape and Surface Morphology

Shape and surface morphology of microspheres were studied using Scanning Electron Microscope (Hitachi S 3700N scanning electron microscope). The samples for SEM study were prepared by lightly sprinkling the formulation on a double adhesive tape stuck to an aluminum stub. The stubs were then coated with gold to a thickness of about 300 A^0 under an argon atmosphere using a gold sputter module is a high vacuum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with a scanning electron microscope. [11]

3. Micromeritic Properties a. Angle of Repose

Flow properties of the microspheres were evaluated by determining the angle of repose and the compressibility index. Static angle of repose was measured according to the fixed funnel and free standing cone method of Banker and Anderson. A funnel with the end of the stem cut perpendicular to the axis of symmetry is secured with its tip at a given height (1 cm), H, above graph paper placed on a flat horizontal surface. The microspheres were carefully poured through the funnel

Table 3: Micromeritic Properties

Formulation Code	Angle of Repose (°)	Bulk Density (g/cc)	Tapped Density (g/cc)	Hausner's Ratio	Carr's Index (%)
M1	22.98±1.35	0.427±0.015	0.495±0.019	1.15± 0.026	13.74±0.78
M2	22.04±1.80	0.429±0.061	0.503±0.082	1.17±0.011	14.71±0.43
M3	21.83±1.37	0.425±0.033	0.486±0.073	1.14±0.015	12.56±0.97
M4	20.86±1.66	0.357±0.020	0.415±0.023	1.16±0.020	13.99±0.15
M5	19.11±1.89	0.420±0.053	0.477±0.038	1.13±0.010	12.36±0.63
M6	18.56±1.88	0.412±0.013	0.465±0.012	1.12±0.005	11.41±0.18
M7	20.18±2.94	0.414±0.011	0.476±0.038	1.14±0.010	12.97±0.24
M8	21.09±1.71	0.431±0.011	0.497±0.015	1.15±0.015	13.20±0.33
M9	21.77±2.16	0.429±0.040	0.491±0.031	1.14±0.015	12.62±0.86

Where n=3 (SD)

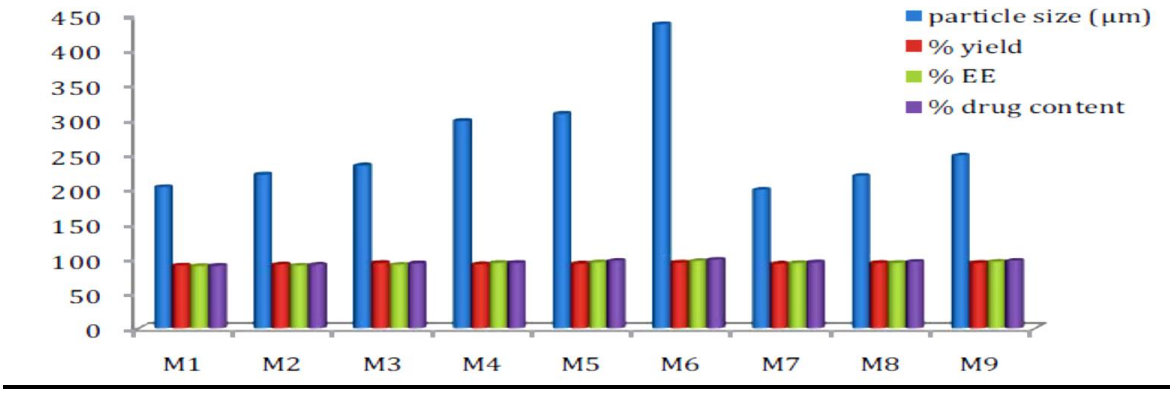

Figure 1: Graph for particle size, % yield, %EE,% Drug content

until the apex of the conical pile so formed just reached the tip of the funnel. [11] Thus, the R being the radius of the base of the microspheres conical pile, H is the height of the pile, θ is the angle of repose,

$$\tan \theta = H/R$$

b. Hausner's Ratio and Carr's Compressibility Index

Accurately weighed microspheres were poured gently through a glass funnel into a graduated cylinder exactly to 10 ml mark. Initial volume was noted. Bulk density and tapped density were noted using tapping method using 10 ml measuring cylinder. The results are showed in the Table 3. They are calculated by using the following formula-

$$Hausner's\ ratio = \frac{TBD}{BD}$$

4.Drug Content Determination, Entrapment Efficiency

mg of microspheres were crushed and dispersed in 10 ml PBS pH 7.4 for 10 min with occasional shaking. The suspension was then centrifuged for 5 min and the supernatant was kept aside. The sediment microspheres were then incubated for 48 hrs with PBS pH 7.4 and the drug concentration was determined spectrophotometrically by UV at 331 nm. [11] The entrapment efficiency, drug loading (n=3) were calculated by using the formulae

% = / ×100

Where, Dcal is the calculated drug content and Dth is the theoretical drug content, respectively.

% = / ×100

Where, Wd and Wm represents weight of drug and weight of microspheres, respectively.

5. *In-Vitro* Drug Release Studies

50 The drug release rate from the microspheres was studied in a medium of changing pH using the dissolution apparatus II at 37±0.5°C with a rotation speed of 100 rpm. A weighed amount of

S. No.	Parameter	Initial	3 Months
1	% Drug content	98.89	98.81
2	% Drug release	95.12	95.10

mesalamine microspheres (equivalent to 100 mg of drug) were added to dissolution medium (0.1N HCl, pH 1.2) for the first two hours. At the end of second hour, the pH of the dissolution medium was raised to 6.8 by the addition of solution composed of 3.75 g of KH_2PO4 and 1.2g of NaOH. At the end of fourth hour pH was raised to 7.4 by adding of phosphate buffer concentrate (2.18 g of KH_2PO4 and 1.46 g of NaOH in distilled water). At predetermined time intervals, 5ml sample was withdrawn, passed through a 0.45 µm membrane filter (Millipore). [11] After appropriate dilutions

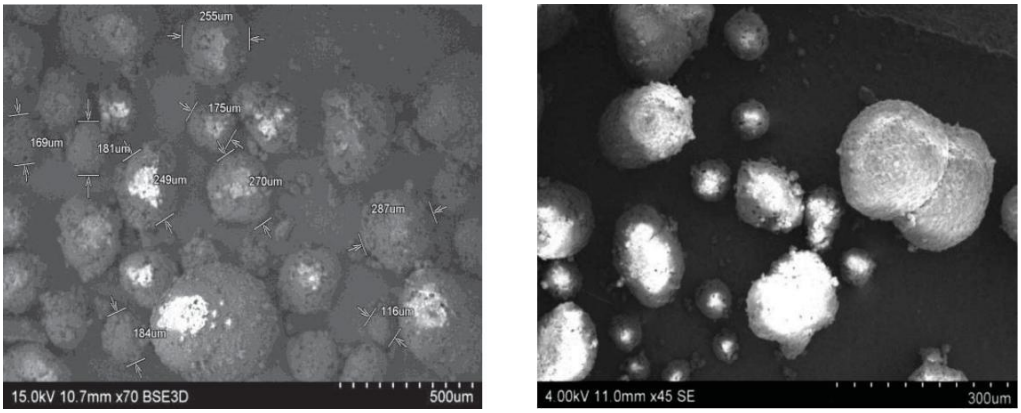

Figure 2: SEM images

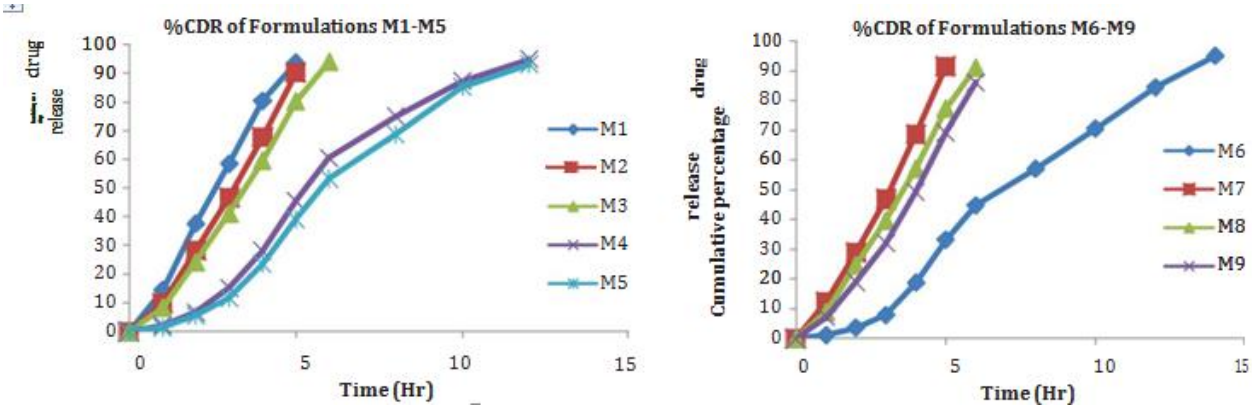

Figure 3: *In-vitro* release graphs of all formulations

Table 4: Stability Studies of Optimized Batch M6

S. No.	Parameter	Initial	3 Months
1	% Drug content	98.89	98.81
2	% Drug release	95.12	95.10

the concentration of drug in samples was analyzed spectrophotometrically at predetermined λ_{max} (1.2 pH-320 nm, 6.8 pH-325 nm, 7.4 pH-331 nm). The initial volume of dissolution medium was maintained by adding 5 ml of fresh dissolution medium after each withdrawal.

6. Release Kinetics [12]

In order to describe the kinetics of the release process of the drug in the different formulations, models were fitted to the dissolution data of formulations using linear regression analysis. In order to study the exact mechanism of drug release from mesalamine microspheres formulation, drug release data was analyzed Zero order kinetics, first order kinetics, Higuchi model, Hixson-Crowell equation, Korsmeyer peppas equation.

7. Stability Studies

A study was carried out to assess the stability of the mesalamine microsphere. Generally the observation of the rate at which the product degrades under normal room temperature requires a long time. To avoid this delay, the principles of accelerated stability studies were adopted. The stability studies were performed using ICH guidelines. The optimized microspheres of M6 were packed in glass container. Stability studies were carried out at 40ºC/75% RH over a period of 3 months. [13] Samples were withdrawn after 3 months and evaluated for change in drug release pattern. The results are shown in Table 4.

RESULTS AND DISCUSSION

Micromeritic properties

The prepared microspheres are evaluated for the micromeritic properties like angle of repose, Hausner's ratio, Carr's compressibility index. The results are tabulated in Table 3.

Particle Size, % Yield, % Entrapment Efficiency, % Drug Content

Result for particle size, % yield, % entrapment efficiency, % drug content is graphically shown in Figure 1.

Shape and surface Morphology

The shape and surface morphology are determined by using Hitachi S3700N Scanning electron microscope. The following images show the photographs of SEM. The microspheres of M6 formulation were subjected to SEM. Different magnifications were used while taking these

photographs. The microspheres of M6 formulation showed spherical shape. The smoothness of the microsphere was found to be increased which may be due to increasing with polymer ratio.

***In-Vitro* Drug Release Studies**

The percentage drug release of mesalamine microspheres M1-M9 were observed at different time periods in 1.2 pH for 2 hours, 6.8 pH for next 3 hours and in 7.4 pH for the next 9 hours. At the end of the study the results of % drug release were found to be M1-93.75%, M2-90.19% i.e. maximum drug release was found to be released in the first 5 hours before reaching the colon. The % cumulative drug release of M3 was 94.16% at the end of 6^{th} hour. For M4 and M5 it was 94.86% and 93.65% respectively at the end of 12^{th} hour. M7 showed a drug release of 91.65% at the end of 5^{th} hour, M7 and M8 showed drug release of 91.32 and 86.21% before reaching the colon. M6 showed maximum drug release i.e. 95.12% at the end of 14^{th} hour in a sustained manner when compared to other formulations.

Release Kinetics

The *in-vitro* studies were fitted into various equations. According to the results mentioned the optimized batch M6 fitted into Hixson Crowell release kinetics with an R^2 value of 0.998 which indicates that the release rate is limiting by the drug particles dissolution rate by erosion release mechanism. And further the n value is > 0.89 indicates Super case II transport.

Stability Studies

The accelerated stability studies were performed for the optimized batch M6 for 3 months at 40°C/75% RH. The results are shown in Table 4. The % Drug content and the % drug release showed negligible results after 3 months showing that the formulation was stable.

CONCLUSION

Microspheres of mesalamine solid dispersion were prepared successfully using the solvent evaporation method. The yield and entrapment efficiency was high for all the formulation prepared. Particle size, entrapment efficiency and production yield were highly influenced by the type of polymer and polymer concentration and also as the concentration of the Eudragit S 100 is increased the microsphere roundness was increased. *In-vitro* dissolution of optimized formulations M6 (95.12%) of Eudragit S100 has the potential to target mesalamine to the colon for the sustained release of drug in the colon. The best fit model for the optimized batch M6 was Hixson Crowell release kinetics with an R^2 value of 0.998 which indicates that the release rate is limiting by the drug particles dissolution rate by erosion release mechanism.

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EVALUATION OF ANTI-QUORUM SENSING ACTIVITY AND ANTI- BIOFILM ACTIVITY OF *PRUNUS AVIUM* FRUIT EXTRACT

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A B S T R A C T

Bacteria inhabit dense, surface-bound communities, termed biofilms, within which they communicate and respond to local cell density through a process known as quorum sensing. Biofilms are estimated to be associated with 80% of microbial infections and the growth of micro-organisms in biofilms can enhance their resistance to antimicrobial agents. Targeting the bacterial communication system (quorum sensing, QS) can be chosen as one of the novel strategy to combat anti microbial resistance. In this work, Prunus avium fruit extract was prepared using sox let extraction. Escherichia Coli culture was maintained on nutrient agar medium and transferred on to L.B broth and bacterial extract is prepared using Orbital shaker incubator. Escherichia Coli Bio film was prepared on cover slips and slides. The intensity of bio film was measured by crystal violet assay method. The extra cellular polysaccharide was extracted. Anti quorum sensing activity of Prunus avium fruit extract was estimated using disk diffusion metod. The anti biofilm activity was measured by crystal violet assay method. In this study Prunus avium fruit extract has shown promising anti quorum sensing activity against Escherichia coli. Prunus avium fruit extract was tested against urinary catheter infected with Escherichia Coli biofim. There is a significant decrease in the intensity of the bio film of Escherichia coli after a period of 3 days.

Key words: Quorum sensing, biofilm, Prunus avium, anti biofilm agents and Escherichia coli

P Neeraja, Ch Sravya, B Ramya bhanu, D Ravali, Evaluation of Anti-Quorum sensing activity and Anti- Biofilm activity of *prunus avium* fruit extract,

1. INTRODUCTION

Biofilm is a group of microorganisms in which microbial cells stick to each other irreversibly (not removed by gentle rinsing) and they often adhere to a surface. . Bacteria exhibit this type of behavior by chemically signaling to one another by a process called quorum sensing[1].Quorum sensing bacteria releases a chemical molecule called auto inducers; these auto inducers modulate gene expression[2]. Biofilms acts as a mechanism towards bacterial resistance[3]. Growth of micro-organisms in biofilms can enhance their resistance to antimicrobial agents. As a consequence antimicrobial therapy often fails to eradicate biofilms from the site of infection. For this reason, innovative anti-biofilm agents with novel targets and modes of action are needed. Biofilms have been found to be involved in variety of microbial infections[4]. An estimate was made that 80% of microbial infections in the body are caused due to biofilms. Infections in which biofilms infect include many common problems such as catheter infections, urinary tract infections, bacterial vaginosis, middle-ear infections, formation of dental plaque, gingivitis, xendocarditis, infections in cystic fibrosis, and infections of permanent indwelling devices such as joint prostheses and heart valves. Plaque is a biofilm formed on teeth[5]. The microorganisms accumulate on teeth and release high concentrations of bacterial metabolites leading to dental disease[6, 7]. *Prunus avium* is commonly called as sweet cherry belonging to the family Rosaceae. Cherries are natural sources of antioxidants, Polyphenols, anthocyanins , sugars, Hydroxycinnamic acids , Anthocyanins and Flavan-3-ols. *Prunus avium* extracts has antimicrobial activity[8,9]. Quorum sensing activity is shown by some plants[10].

2. MATERIALS AND METHODS

Preparation of *Prunus avium* fruit extract:

Extraction procedure: Fresh cherry fruit of about 100g is taken and its seed and peel is removed. Then the fruit is soaked in methanol for about 24 hours. The soaked cherry fruit is soxhlet with methanol for about 6 hours at 600C. Remove the mixture from soxhlet and evaporate the mixture to evaporate methanol. Centrifuge the mixture at 5000 rpm for 3 minutes and collect the supernatant liquid. The extract is temporarily stored in refrigerator.

Preparation of media:

Preparation of LB Broth: Triptone (10gm/ml), Yeast extract (5gm/ml), Sodium chloride (10gm/ml) is taken into a1000ml conical flask. About 500ml of water is taken and slowly added in to the flask by shaking the flask until clear liquid is formed. The medium is sterilized in a autoclave.

Preparation of Agar Medium: Peptone (6gm), Beef extract (1.5gm), Yeast extract (3gm), Agar (15gm) are taken into a conical flask and water (1000ml) is added slowly in to the flask by stirring. A clear solution is formed and the medium is sterilized.

Organism and culture maintenance:

Escherichia coli culture was maintained on nutrient agar medium. The slants were incubated at 37°C for maximum growth. The fully grown slants were maintained at 4°C and were sub cultured for every 2 weeks.

Preparation of bacterial extract:

It is carried out in 250ml conical flasks containing 100ml of LB Broth. To the broth test microorganism i.e. *Escherichia coli* was added separately in two flasks. The inoculated broth is kept in orbital shaker incubator for 72 hours so that the microbes are evenly distributed in the broth. The bacterial extract is now used for preparation of biofilm.

Preparation of biofilm:

A clean groove slide is taken and immersed in a container of alcohol and sterilized by flaming. The slide is allowed to cool for a few seconds and then one or two drops of inoculated broth is added. The groove slide is covered with an unbreakable cover slip which is also dipped in a container containing alcohol so as to sterilize. In the same manner five or six slides are prepared and placed in a incubator for 48 hrs under aseptic conditions. After 48 hrs of incubation, using forceps which is sterilized by dipping in a container containing alcohol and by flame the cover slips are removed carefully without breaking and placed on a sterile filter paper. The biofilm on the cover slips should appear as a slimy layer coating the under surface of the slide. Lifting rapidly may disturb the biofilm causing large sections to slough off the slide. In laminar air flow cabinet, agar medium is poured in petriplate and allow it to solidify. The biofilm formed cover slips are retained on agar medium and incubated again for 48 hours for more good results.

Alternative method:

Agar medium which is sterilized in autoclave is poured in petriplate and allow it to solidify in air flow cabinet. To the above medium sterilized cover slips are inserted, see that there is no air bubbles formation. Test organisms i.e. *Escherichia coli* was poured to different petriplate and incubated for 48 hours.

Detection of bio film:

The ability of biofilm producing microbes was tested by staining the heat fixed cells with crystal violet. The petriplate containing cover slips and slides were washed after incubation, three times with sterile distilled water to remove loosely associated cells. The plates were air dried and then dried in oven at 60°C for 45 min. Following drying, they were stained with 100 µL of 1%crystal violet and incubated at room temperature for 15 min after which the plates were washed 5 times with sterile distilled water to remove unabsorbed stain[11, 12].

Anti quorum sensing activity of *Prunus avium* fruit extract:

Disk diffusion assay is selected for measurement of potential anti-quorum sensing activity.

Diffusion assay:

The Disk diffusion assay is an assay used to evaluate anti- QS activity by evaluating zones of inhibition around the disk. Each extract (50 µL) was loaded onto sterile disks (6 mm diameter), placed onto prepared LB plates containing biofilm formed cover slips of *Escherichia coli*. Plates were incubated in an incubator at 30°C overnight and anti-QS activity was detected by a ring of colorless, but viable cells around the disk. Measurements were made from the outer edge of the disks to the edge of the zones of anti-QS activity. Results were determined by observing the growth inhibition colorless ring diameter, opaque circle around the extract loaded disk.

Anti bio film activity of *Prunus avium* fruit extract:

Anti bio film activity of *Prunus avium* fruit extract of *Escherichia coli* was qualitatively estimated by a method described by Xiao et al. 40 µL of exponentially growing cells were dispensed in wells prepared in cell culture plates. *Prunus avium* fruit extract was added to the wells and incubated for 24 h at 37°C. The concentrations of extracts were ranged from 10 to100 µg/mL. The medium without extracts was used as the non-treated control. After incubation, media and unattached cells were decanted and washed with Phosphate Buffer Saline (PBS). Then the plate was air dried and it is stained with 0.1% (v/w) Crystal Violet (Sigma-Aldrich, Germany). 1000 µl of 95% (v/v) ethanol was added to each well for 1 h to release the stain. Cells were transferred to a new test tube for spectrophotometric analysis (OD570 nm)[13]. Inhibition mediated reduction of biofilm formation was calculated by the following formula % of inhibition = OD in control –OD in treatment ×100 OD in control

Effect of *Prunus avium* fruit extract on Bacterial (*Escherichia Coli)* Bio film in Urinary Catheter

About 5 mL of urine sample was injected into catheters to induce bio film formation on the internal surface of the catheter. *Escherichia coli* cultures were incubated on a ,freshly prepared sterile nutrient broth and injected into catheters. The catheters were stoppered with the catheter caps and incubated at 25°C for 120 hrs[14].

Coating of catheter with the plant extracts: The catheters were immersed in warm water bath. This leads to tenderness of the catheter and allowed for easy coating of the inner surface layer of the catheters with the plant extracts. 20ml of the plant extract was injected into catheter. The catheters were placed such that the both end of the tubes were out of the warm water. This is then allowed for quick coating of the plant extract and evaporation of the solvent (ethanol) that was used to prepare the extract[15].

3. RESULTS

Preparation and detection of bio film of *Escherichia coli*

Bio film of *Escherichia coli* was prepared in sterile cover slips. In laminar air flow cabinet, agar medium is poured in petriplate and allow it to solidify. The bio film formed cover slips are retained on agar medium and incubated for 48 hours for more good results. In another method, to agar medium containing cover slips and slides test organisms i.e. *E.coli* was added and incubated for 48 hours. The ability of biofilm producing microbes was tested by staining the heat fixed cells with crystal violet. mAs *Escherichia coli* is a gram negative bacteria its cell wall consists of less amount of peptidoglycan. So it has increased permeability and increased pore size. When crystal violet was added to *E.coli* cell culture, blue color diffuses into the cell. Blue colored stained cells were observed under projection microscope (Figure. 1).

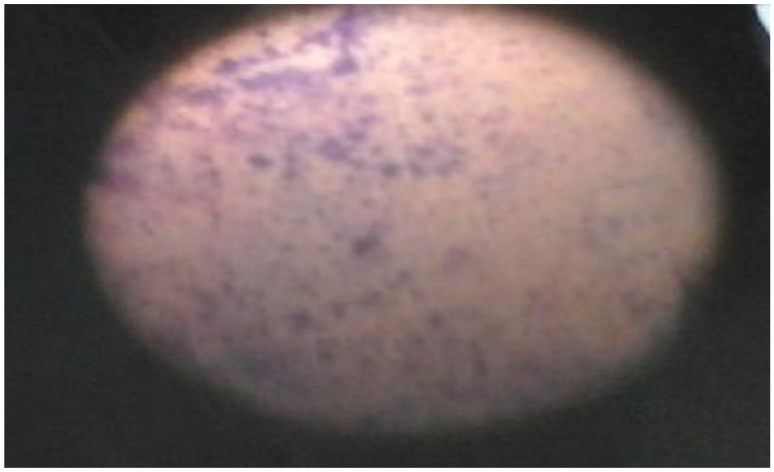

Figure1: Detection of bio film of *Escherichia coli*

Anti quorum sensing activity of *Prunus avium* fruit extract against *Escherichia coli* bio film was determined by disk diffusion assay (Figure.2). In this study *Prunus avium* fruit extract has shown promising anti quorum sensing activity against *Escherichia coli*.

Figure2: Anti quorum sensing activity of *Prunus avium* fruit extract

Anti bio film activity of *Prunus avium* fruit extract:

Bio film inhibition studies carried out using plant extracts have successfully inhibited bio film formation of *E.coli*. The plant extract inhibited bio film as dose dependent manner. The results were presented in Table 1.

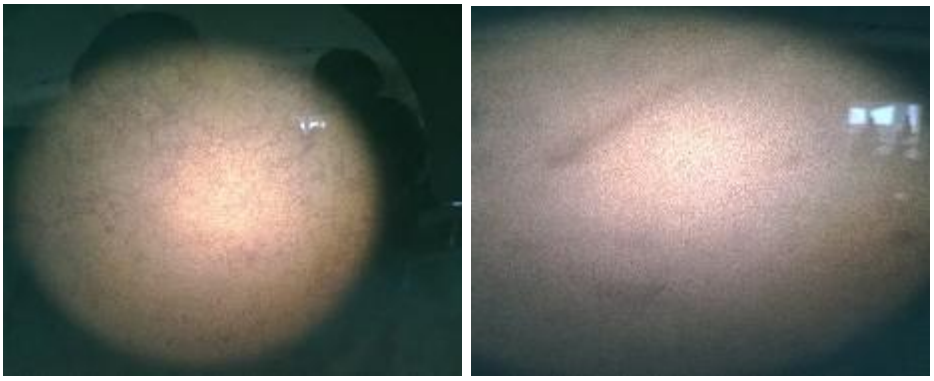

Figure.3 Anti bio film activity of *Prunus avium* fruit extract in Crystal violet assay

Table 1: Measurement of Anti bio film activity of *Prunus avium* fruit extract:

S. No	Total bio film	% inhibition
Control	0.53 ± 0.02	0
10µg/ml	0.51 ± 0.01	7.57
25 µg /ml	0.43 ± 0.01	22.87
50 µg /ml	0.31 ± 0.02	47.51
70 µg /ml	0.29 ± 0.003	58.78

The effect *Prunus avium* fruit extract in Prevention of *Escherichia Coli* bio film in Urinary Catheter.

Prunus avium fruit extract was injected into the catheter coated with bio film of *Escherichia coli* and observed for a period of 15 days. There is a significant decrease in the intensity of the bio film of *Escherichia coli* after a period of 3 days (Fig 4, Fig 5).

Figure 4*: Escherichia Coli* bio film in Urinary Catheter.

Figure 5: Effect *Prunus avium* fruit extract in prevention of *Escherichia Coli* bio film in Urinary Catheter.

4. DISCUSSION

Microorganisms undergo various changes during their transition, these changes leads to formation of biofilm16. Biofilms are resistant to disinfectants when they are present on surfaces17. Many microbial species can form biofilms e.g.

Escherichia coli strains18, 19. *Escherichia coli* cause urinary catheter infections.

In this work, *Prunus avium* fruit extract was prepared using sox let extraction. *Escherichia coli* culture was maintained on nutrient agar medium and transferred on to L.B broth and bacterial extract is prepared using Orbital shaker incubator. *Escherichia Coli* bio film was prepared on cover

slips and slides. The intensity of bio film was measured by crystal violet assay method. The extra cellular polysaccharide was extracted. Anti quorum sensing activity of *Prunus avium* fruit extract was estimated using disk diffusion method. The anti biofilm activity was measured by crystal violet assay method. In this study *Prunus avium* fruit extract has shown promising anti quorum sensing activity against *Escherichia coli.Prunus avium* fruit extract was tested against urinary catheter infected with *Escherichia coli* biofilm. There is a significant decrease in the intensity of the bio film of *Escherichia coli* after a period of 3 days.

5. CONCLUSION

In summary, the selected *Prunus avium* fruit extract may have a potential, and serve as important as medicinal plants in inhibiting quorum sensing of bacteria and further acts as a anti biofilm agent. Further study has to be done to identify the active compounds of the identified plant extract and mechanism of actions of quorum sensing by the active compound.

6. ACKNOWLEDGEMENT

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PHYTOCHEMICAL INVESTIGATION AND ANTIHELMINTIC ACTIVITY OF *OPERCULINA TURPETHUM* ROOTS

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STRACT

The present study was aimed at the investigation of the roots of the traditional Indian medicinal plant *Operculina turpethum* for pharmacologically active chemical constituents and *in vitro* evaluation of the fraction (or) extracts that shown to contain maximum constituents for anthelmintic activity against adult Indian earthworms (*Pheretima posthuma*) using albendazole as the reference standard. The collected and authenticated roots were dried under shade and extracted with water, ethanol, and ethyl acetate by maceration. The obtained extracts were investigated for the presence of various biologically active ingredients by qualitative methods. Ethanolic and ethyl acetate extracts that were shown to possess maximum constituents were tested for anthelmintic activity by measuring parameters such as time taken for paralysis and death of the worms. Results were compared with that were obtained with albendazole. From the results, it was found that the ethanolic extract of the roots taken for the study possesses significant activity at the concentrations of 150 mg/ml. However, more study is recommended for further investigation in this regard.

KEY WORDS: Albendazole, Anthelmintic activity, Extracts, *Operculina turpethum*, Phytochemical investigation, Roots

Pulipaka Shankaraiah, Anasuri Santhosh, M. Ravi Kumar, Bharat Bhusan Mahapatra**,** Phytochemical investigation and AntiHelmintic activity of *operculina turpethum* roots,Journal of pharmacy Research,2018,12(1),90-95

INTRODUCTION

The human being appears to be afflicted with more diseases from the early ages, taking advantages of plants growing around them to alleviate their sufferings from injury or disease with hopes for remedies in chronic diseases generated new enthusiasm in the research workers to develop herbal medicines.

Herbal medicine offers a greater scope for the future treatment of various pathological conditions.

The effectiveness of medicinal plant lies in the varying complex chemical substances such as alkaloids, glycosides, corticosteroids, and essential oils which are the starting material for a vast number of synthetic drugs.[1,2]

Helminthiasis is one of the most important animal diseases worldwide that can cause heavy production losses in grazing animals. The disease is prevalent all over the world, especially in developing countries, and is always associated with poor management practices and inadequate and

inappropriate control strategies. An integrated approach is required for the effective control of helminths which includes strategic and tactical use of anthelmintics which remains the cornerstone to this end and careful management of grazing lands including control of stocking rates and appropriate rotation strategies.

Role of vaccinations is also vital for the control of various parasitic diseases as in the case of lungworms. However, various problems have emerged with the use of anthelmintics, and among them, resistance against various species of helminthes is of utmost importance to different anthelmintic compounds and classes, as well as chemical residue and toxicity problems.

In addition, recognition of the antigenic complexity of parasites has slowed vaccine development. For these various reasons, interest in the screening of medicinal plants for their anthelmintic activity remains of great scientific significance despite extensive use of synthetic chemicals in modern clinical practices worldwide.[3] The plant kingdom is known to provide a rich source of botanical anthelmintics, antibacterials, and insecticides.[4]

A number of medicinal plants have been used to treat parasitic infections in man and animals. However, their scientific evaluation as compared to commercial anthelmintics is limited.[5-10]

Operculina turpethum, convolvulaceae family, is a well-known traditional Indian medicinal plant various parts of which were traditionally claimed for the treatment of snake, scorpion bites, as laxative, anti-anemic, anti hypercholesterimic, for gout, fevers, and leprosy conditions. In the modern scientific era, these were reported to possess nephroprotective, hepatoprotective, anticancer, antioxidant, antimicrobial, antiulcer, antidiabetic, antiarthritic, analgesic, antidiarrheal, anti-inflammatory, and laxative effects. These were also scientifically reported for the presence of various bioactive constituents of pharmaceutical significance such as alkaloids, glycosides, coumarins, steroids, volatile oils, carbohydrates, and turpethinic acids.[11]

The present study was aimed at the investigation of the roots of *O. turpethum* for the presence of various pharmacologically active chemical constituents and *in vitro* evaluation of the selected extracts that were shown to possess maximum constituents for anthelmintic activity using adult Indian earthworms comparing with albendazole standard drug. It is probably the first report in this regard.

MATERIALS AND METHODS

Collection of Plant Materials

O. turpethum (L.) Silva Manso plant is a common weed throughout Godavari valley of Telangana.

O. turpethum (L.) Silva Manso plant material collected from local areas of Warangal, Telangana. Its parts were botanically authenticated at the Department of Botany, Kakatiya University, Warangal, Telangana, India.

Preparation of Plant Material

O. turpethum and roots were washed under tap water and were efficiently dried under shade for about 1 week and protected from deterioration. The shade dried and roots were grinded made into powder with the help of a laboratory mixer. These were efficiently dried under shade for about 1 week and protected from deterioration and then grinded and made into powder.

Extraction/Maceration

The root material was weighed (100 g) and using successive solvent extraction process (Soxhlet apparatus) with ethyl acetate, ethanol, and water for 6 h. After completion of Soxhlation process, the liquid extract was collected and concentrated under reduced pressure below 50°C until a soft mass obtained, it was dried and kept in a desiccators.

The percentage of extractives was calculated with reference to the air-dried drug.

Chemicals

All the chemicals and reagents used in the work are of analytical grade and were procured from well-recognized sources in Hyderabad.

Animals

Adult Indian earthworms required for *in vitro* evaluation of the activity were procured from the local areas of the institutions and were maintained under standard conditions of living as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals during the coursework.

PHYTOCHEMICAL EVALUATION

A systematic and complete study of crude drugs should include an investigation of both primary and secondary metabolites derived as a result of plant metabolism. The different qualitative chemical tests are to be performed for establishing profile of a given extract for its nature of chemical composition.

Qualitative phytochemical screening was carried out for the presence of various phytochemicals in the obtained extracts as per the procedure by Kokate *et al*.[12]

Chemical Investigation of Crude Extracts of *O. turpethum*

The extracts of *O. turpethum* subjected to chemical investigations to detect the presence of various phytoconstituents in the crude extracts. The following chemical tests and thin-layer chromatography study were performed for extracts.

Test for alkaloids

About 50 mg of the extract was stirred with few ml of dilute hydrochloric acid and filtered. The filtrate was tested with various reagents for alkaloids as follows:

Dragendorff's test

To 2–3 ml of filtrate, added few drops of Dragendorff's reagent. Orange–brown precipitate indicates the presence of alkaloids.

Mayer's test

To a few ml of filtrate, 2–3 drops of Mayer's reagent was added along the sides of the test tube. The formation of white or creamy precipitate indicates the presence of alkaloids.

Wagner's test

To a few ml of filtrate, 2–3 drops of Wagner's reagent was added along the sides of the test tube. The formation of reddish-brown precipitate indicates the presence of alkaloids.

Hager's test

To a few ml of filtrate, 2–3 drops of Hager's reagent was added along the sides of the test tube. The formation of yellow precipitate indicates the presence of alkaloids.

Test for carbohydrates

Molisch's test

To 2–3 ml of aqueous extract, added few drops of α-naphthol solution in alcohol, shaken and added concentrated H2SO4 from sides of the test tube. The formation of violet ring at the junction of two liquids indicates the presence of carbohydrates.

Test for reducing sugars

Fehling's test

To 2-3 ml of the test solution, added 1 ml each of Fehling's A and 1 Fehling's B solutions. Heated in boiling water bath for 5–10 min. Formation of yellow, then brick-red precipitate indicates the presence of reducing sugars.

Benedict's test

Equal volume of Benedict's reagent and test solution was mixed in test tube. Heated in boiling water bath for 5 min. Soon appears green, yellow, or red depending on the amount of reducing sugar present in test solution.

Test for monosaccharide

Barfoed's test

Equal volume of Barcode's reagent and test solution was mixed. Heated for 1–2 min in boiling water bath and cooled, formation of red precipitate indicates the presence of sugars.

Test for non-reducing polysaccharides (starch)

Iodine test

3 ml test solution and few drops of dilute iodine solution were mixed. Blue color appears; it disappears on boiling and reappears on cooling.

Test for proteins

Millon's test

To 2 ml of filtrate, two drops of Millon's reagent was added and observed for the formation of a precipitate. Formation of a white precipitate indicates the presence of proteins.

Biuret test

To 3 ml of filtrate, two drops of 4% NaOH was added and treated with two drops of 1% $CuSO_4$ solution. Formation of pink color indicates the presence of proteins.

Test for amino acids

Ninhydrin test

To 3 ml of filtrate, three drops of 5% Ninhydrin reagent was added and heated in boiling water bath for 10 min. Formation of a characteristic purple color indicates the presence of amino acids.

Test for steroids

Liebermann–Burchard's test

A small amount of extract was dissolved in chloroform. To this, 1–2 ml of chilled acetic anhydride was added and mixed well. Then, 2–3 drops of chilled concentrated H_2SO_4 was added along the walls of test tube. An array of colors indicates the presence of phytosterols.

Salkowski's test

The extract in chloroform was treated with a few drops of concentrated H_2SO_4, shaken well and allowed to stand. The formation of yellow and reddish-brown colored layers indicates the presence of triterpenes and steroids, respectively.

Test for tannins and phenolic compounds

5% ferric chloride test

About 50 mg of extract was dissolved in 2 ml of distilled water, added two drops of neutral 5% ferric chloride solution and observed for coloration. Formation of blue, black, green, and violet

color indicates the presence of phenolic compounds. Test solution treated with few drops of ferric chloride solution gives deep blue–black color which indicates the presence of tannins.

Lead acetate test

About 50 mg of the extract was dissolved in 2 ml of distilled water, and to this, 3 ml of 10% lead acetate solution was added. Formation of bulky white precipitate indicates the presence of phenolic compounds.

Test for glycosides

Borntrager's test

About 50 mg of extract was hydrolyzed with 2 ml of concentrated hydrochloric acid for 2 h on a water bath and filtered. To 2 ml of filtrate hydrolysate, 3 ml of chloroform was added and shaken, chloroform layer was separated, and 10% ammonia solution was added. Formation of pink color indicates the presence of anthraquinone glycosides.

Test for saponin glycosides

Foam test

About 50 mg of extract was dissolved in 2 ml of alcohol, diluted with 20 ml of distilled water, and shaken for 15 min in a graduated cylinder. A layer of stable foam indicates the presence of saponin glycosides.

Test for flavonoid glycosides

Shinoda's test

To 50 mg of the extract, 5 ml of 95% ethanol, 2–3 drops of concentrated HCl, and 0.5 g of magnesium turnings were added. Formation of pink color indicates the presence of flavonoids. 50 mg of the extract was dissolved in 2 ml of alcohol, and to the extract, increasing amount of sodium hydroxide was added. It shows yellow coloration, which decolorizes after addition of an acid if flavonoids are present.

Test for coumarins glycosides

a. Coumarin glycosides have aromatic odor.
b. Alcoholic extract, when made alkaline, shows blue or green fluorescence.

Antihelmintic Activity

The method of Ghosh et al.[13] with minor modifications was adopted for the evaluation of the anthelmintic activity of the plant extract. It was done using adult Indian earthworms Pheretima posthuma. Earthworms were divided into eight groups (5 each). The first Group (I) given the standard drug albendazole at a dose level of 10 mg/ml. Groups of II to VI received different doses of extracts says 10 mg/ml, 15 mg/ ml, 20 mg/ml, 25 mg/ml, 30 mg/ml, and 35 mg/ml, respectively. Observations were made for the time taken to cause paralysis and death of individual worm for 2 h. Paralysis was confirmed when the worms did not revive even in normal saline water. Death was concluded when the warms lost their lost motility followed by fading away of their body color.

RESULTS

Yield of the Extracts

The percentage of the yield of different extracts of the roots of O. turpethum was presented in Table 1. From Table 1, maximum yield was found to be ethanolic extract (EE), followed by ethyl acetate and aqueous extracts.

Preliminary Phytochemical Analysis

The results of phytochemical investigation of various extracts are represented in Table 2. From Table 2, it was found that the EE of the plant roots was found to contain maximum biologically

active constituents followed by ethyl acetate extract (EAE). Therefore, ethanolic and EAE were selected for the evaluation of antihelmintic activity. **Antihelmintic Activity**

The results obtained for the EE EAE extracts of the plant roots were represented in Tables 3 and 4.

All the values were expressed as a mean ± standard deviation. Statistically analyzed using ANOVA method and significant difference was found among the values* ($P < 0.05$).

All the statistical analysis was done using Graphpad Prism software.

From Tables 3 and 4, it was found that the ethanolic followed by EAE taken for the investigation of anthelmintic activity possesses dose-dependent and significant activity when compared with standard albendazole (10 mg/ml) at the concentration of 150 mg/ml with respect to the parameters evaluated such as time taken for paralysis and death of the worms.

DISCUSSION

Several natural products from plant origin like to say, Gmelina arborea roots and Carissa carandas roots were previously reported scientifically, for the presence of various phytoconstituents such as flavonoids, phenolic compounds, and alkaloids were also reported scientifically for their anthelmintic activities in different pharmacological screening models.[14-16] The roots of the plant O. turpethum taken in the current investigation were also found to contain similar biologically active constituents and also found to possess anthelmintic activity in the tested conditions. These constituents may be

Table 1: Yield of different extracts

Solvent	Percentage of extract (w/w)
Alcohol	2.8
Water	2.2
Ethyl acetate	2.5

Table 2: Phytochemical investigation of different extracts

Test	Extract		
	Ethanolic	Ethyl acetate	Aqueous
Alkaloids	+	+	−
Flavonoids	+	+	−
Glycosides	+	+	+
Saponins and coumarins	+	−	−
Steroids	+	+	−
Tannins and phenolic compounds	+	+	−
Carbohydrates	+	−	+
Proteins and amino acids	+	−	−

Table 3: Anthelmintic activity of ethanol extract of *O. turpethum*

Groups	Treatment	Concentration	Time taken for	Time taken for

		used (mg/ml)	paralysis (min) (X=SD)	death (min) (X=SD)
1	Vehicle normal saline	-	-	-
2	Standard (albendazole)	10	15.50±0.25*	20.54±0.2
3	EE 1	10	56.8±0.5	72.3±0.5
4	EE 2	20	52.2±0.5*	68.4±0.4
5	EE 3	40	36.3±0.2*	49.4±0.1
6	EE 4	100	30.2±0.3*	45±0.4
7	EE 5	150	19.6±0.3	26.8±0.3

SD: Standard deviation, EE: Ethanolic extract, *O. turpethum: Operculina turpethum*

Table 4: Antihelmintic activity of EAE

Groups	Treatment	Concentration used (mg/ml)	Time taken for paralysis (min) (X=SD)	Time taken for death (min) (X=SD)
1	Vehicle normal saline	-	-	-
2	Standard (albendazole)	10	14.50±0.50*	21.34±0.5
3	EAE 1	10	60.9±0.7	72.5±0.5
4	EAE 2	20	58.2±0.7*	69.6±7
5	EAE 3	40	39.4±0.6*	55.7±0.7
6	EAE 4	100	34.4±0.6*	48±0.7
7	EAE 5	150	24.4±0.5	29.7±0.8

EAE: Ethyl acetate extracts, SD: Standard deviation

responsible for the pharmacological potential of the plant product. It also scientifically proved certain traditional claims of the plant. It is probably the first report in this regard.

CONCLUSIONS

Among the aqueous, ethyl acetate, and EE of the roots of *O. turpethum* taken for the investigation, the ethanolic followed by EAE was found to possess maximum pharmacologically active chemical constituents and also exhibited significant anthelmintic activity against adult Indian earthworms that are comparable with standard drug albendazole.

However, more investigation in this regard is recommended for isolation and characterization of the specific lead chemical constituents responsible for the biological activity of the extracts and broad evaluation using different models and animals.

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ANTI-ANXIETY ACTIVITY OF *TRADESCANTIA SPATHACEA* ASSESSED USINGDIFFERENT EXPERIMENTAL ANXIETY MODELS

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ABSTRACT

The point of present examination was to investigate the anti-anxiety activity of hydroalcoholic extracts of *Tradescantia spathacea* utilizing different animal models (elevated plus maze, open field test, light and dark test and social interaction test) of anxiety in mice. Diazepam (0.5 mg/kg) was utilized as the standard and measurement of hydroalcoholic extract of *T. spathacea* (50, 100 and 200 mg/kg) was chosen according to OECD rules. Results recommended that concentrate of *T. spathacea* at 100 and 200 mg/kg dose produced anti-anxiety effects almost similar to diazepam and at 50 mg/kg dosage did not create against anti-anxiety activity on any of the paradigm used. Additionally ponders are expected to recognize the anxiolytic mechanism(s) and the phytoconstituents responsible for the observed central effects of the hydroalcoholic extract of *T. spathacea*.

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INTRODUCTION

Anxiety affects simple fraction of the whole population worldwide and has become a crucial space of analysis
interest in pharmacology throughout this decade. [1] Benzodiazepines are the most important category of compounds utilized in anxiety and that they have remained the foremost unremarkably prescribed treatment for anxiety. [2] However, the belief that benzodiazepines gift a slim margin of safety between the anxiolytic impact and people inflicting unwanted aspect effects has prompted several researchers to judge new compounds within the hope that different anxiolytic medicine can have less undesirable effects. [3] The popularity of anxiolytic effects of non-benzodiazepine azapirones agents, which act as 5-HT1A partial agonists, like buspirone, gepirone and ipsapirone and their therapeutic role in clinical anxiety and mood disorders has any targeted attention on the 5-HT1A receptor. [4] Though the azapirones move with different neurochemical systems, like the dopaminergic and noradrenergic, they show nanomolar affinity for 5-HT1A receptor sites. [5] However, the anxiolytic effects of azapirones follow a time course determined with antidepressants wherever therapeutic effects are delayed for 3-4 weeks, that is in contrast to the speedy effects determined with anxiolytic drug anxiolytics. [6] Thus, there's a requirement of strong anxiolytic compounds that have lesser aspect effects than benzodiazepines and additional immediate onset of action than presently out there 5-HT1A receptor acting medicine. [7] *Tradescantia spathacea* Swartz (syn. Rhoeo discolor L. H´er Hance, Rhoeo spathacea (Swartz) Stearn) is a plant of India that is in use in traditional medicine. This plant belongs to the Commelinaceae family. [8] In the Southeastern of Mexico, it is known as “Maguey Morado” (Purple Maguey) and the decoction of the leaves is daily free-consumed as curative of cancer, without existing scientific evidence of such property. [9] It is known that the aqueous extract of *T. Spathacea* blocks the antiadrenergic action of bretylium [10] and is contraceptive in rats. [11] The extracts of *T. Spathacea* have been incorporated in
cosmetics to improve the appearance of skin. [12] Some chemicals detected in *T. Spathacea* are flavonoids, anthocyanins, saponins, carotenoids, waxes, terpenoids and coumarinic and steroidal compounds. [13, 14] On the other hand, *T. Spathacea* ethanolic crude extract evaluated in an *in-vitro* system, showed antioxidative activities [15] and antimicrobial properties. [16] Due to the absence of scientific reports *in-vivo* that
corroborate the anxiolytic activity property of *T. Spathacea*, it is evident the importance of the exploration of this plant. They additionally assessed the spontaneous activity and neuromuscular

coordination. Other than this, no model(s) for anxiety (except EPM) has been used for further evaluation of anxiolytic activity of *T. Spathacea* extract, to our knowledge. The aim of the present study was to explore the anti-anxiety activity of hydroalcoholic extract of *T. Spathacea* totally different animal models (EPM, open field (OF) test, light and dark test and social interaction test) of anxiety in mice.

MATERIALS AND METHODS

Animals

Swiss albino mice (males; 20–25 g) were used in the present study. Divided into 5 groups of 6 animals per cage were used. Animals were maintained under standard laboratory aseptic conditions (12-h light/dark cycle, 24 hrs). The food in the form of dry pellets and water is provided *ad libitum*. The animals were acclimatized to the laboratory conditions before experiments. Experimental protocol was approved by Institutional Animal Ethics Committee. Care of the animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Government of India. Experiment protocol was approved by Institutional Animal Ethics Committee (Reg No: 1648/PO/A/12/CPCSEA).

Plant Material

The plant *Tradescantia spathacea* was collected within the month of Feb. 2017 from medicinal gardens of Geethanjali college of Pharmacy in Cheeryal, Hyderabad, Telangana State, India. The plant specimen was identified by Prof. Rana Kausar, Dept. of Botany, Osmania University, Hyderabad, Telangana State, (voucher specimen no. 0364).

Preparation of Hydroalcoholic Extract

T. spathacea plant was collected, washed thoroughly in water, chopped and air dried at 35–40°C for every week. Dried whole plant was pulverized in an electric grinder to obtain a fine powder. The powder obtained was defatted with petroleum ether and successively extracted with 70% ethanol by employing a soxhlet apparatus. Extract was filtered, concentrated under reduce pressure and dried.

Drugs

Diazepam hydrochloride (Calmpose injection, Ranbaxy Laboratories, Gurgaon, India) was used as a reference drug. It was diluted with saline to the required strength before use. Different concentrations of the *T. spathacea* extract were prepared by serial dilution from a stock solution of 100 mg/ml of the extract in sterile water. All the solutions were prepared freshly on test days and administered intraperitoneally (i.p.) in a very volume of 0.1 ml/10 g body weight of mice.

Elevated plus Maze Test

The plus maze apparatus consisted of two open arms, measuring 16 × 5 cm and two closed arms, measuring 16 × 5 × 12 cm, connected to a central platform (5 × 5 cm). The maze was elevated to a height of 25 cm above the floor. Each mouse was placed individually at the center of elevated plus maze with its head facing toward an open arm and observed for 5 min to record the number of entries into open arm, closed arm and time spent in each arm. [17, 18] In EPM test, the percent time spent on the open arms (100×open/total time) was calculated for each animal.

Light and Dark Box Test

The apparatus consisted a rectangular box (45 × 27 × 27 cm), partitioned into two compartments connected by a 7.5 × 7.5 cm opening in the wall between compartments. An animal was placed in the center of the light compartment and was observed for 5 min for the time spent in open (white/light) compartment. [19] Percent time spent in the light compartment was determined as follows: % = (100×open/total time) (5 min observation time).

Open Field Test

The Open Field Test test, which provides simultaneous measures of locomotion, exploration and anxiety, was used for this study. The open field is a 400 × 400 × 300 mm arena with thin black stripes painted across the floor, dividing it into 16 quadratic blocks. Mouse was placed in the center of arena and an observer quantified the spontaneous ambulatory locomotion of each mouse for 5 min. During this period, the number of squares crossed and number of rearing were measured. [20]

Social Interaction Test

The social interaction arena was an open topped box (22 × 15 × 12 cm). Mice were isolated for 1 h before the test. After introduction to the test arena, the mice were observed for cumulative time spent in genital investigation, sniffing a partner, following, grooming, kicking, biting, wrestling, climbing over and under, neck licking and boxing. [21]

Experimental Protocol

Experimental animal groups used in the present study consisted of six mice in each group. All the instruments

used in present study were fabricated from local market as per the standard dimensions available from scientific research. Mice were exposed to EPM and light–dark test for normal duration (5 min), sufficient to assess the anxiety levels in rodents. [22] Behavioral tests were performed in independent groups of mice. Drugs were administered 30 min before the evaluations in the apparatus. Doses of ethanolic extract of *T. spathacea* (50, 100 and 200 mg/kg) were selected on the basis of acute toxicity study according

to OECD guidelines (up and down method) and the dose of diazepam was 0.5 mg/kg. The apparatus was thoroughly cleaned using 5% ethanol before placing each mouse in the cage.

Statistical Analysis

All the results were expressed as Mean□}SEM. Data were analyzed by analysis of variance (ANOVA) in Origin Pro package, version 8.5. $P<0.05$ was considered as significant.

RESULTS

Elevated Plus Maze Test

Administration of diazepam (0.5 mg/kg) significantly increased the amount of time spent in the open arms and the percentage of open arm entries ($P<0.001$) compared to saline-treated group (Figure 1a). Hydroalcoholic extract of *T. Spathacea* at 100 mg/kg ($P < 0.01$) and 200 mg/kg ($P<0.05$) significantly increased the time spent in the open arms. Entries in the open arms increased significantly at 100 mg/kg ($P<0.01$) and 200 mg/kg ($P<0.01$) (Figure 1b). Plant extract at 50 mg/kg had no significant effects on anym of the parameters that were measured on the EPM.

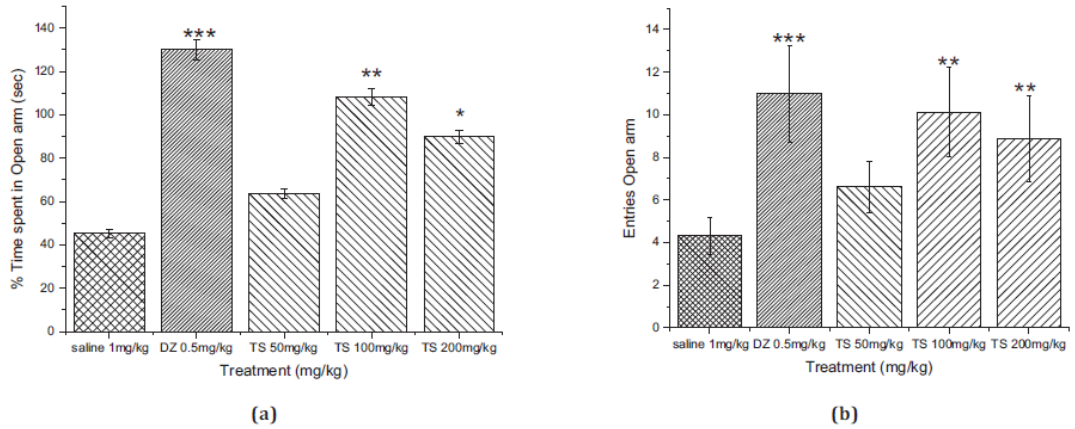
Light and Dark Box Test

Diazepam (0.5 mg/kg) significantly increased the time spent in light compartment ($P<0.001$) compared to salinetreated group (Figure 2). Significant increase in the time spent in the light compartment ($P<0.01$) was seen with administration of 100 and 200 mg/kg of hydroalcoholic extract of *T. spathacea* compared to saline-treated group (Figure 2). Plant extract at 50 mg/kg did not produce any significant effects that were measured by light and dark box test.

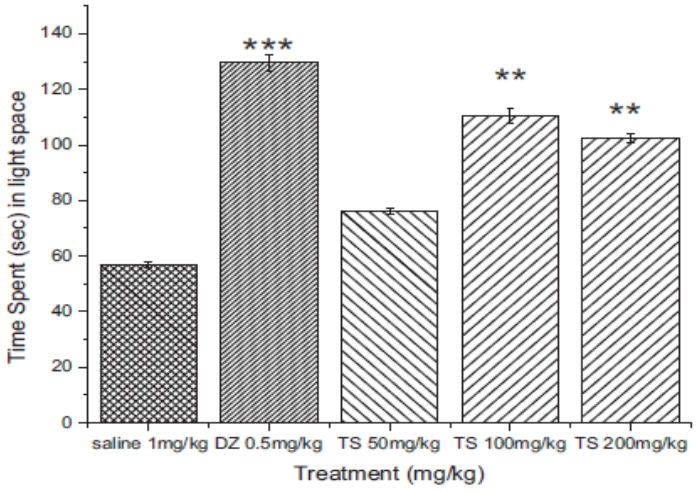
Open Field Test

Treatment with the plant extract at 200 mg/kg showed anxiolytic activity in this paradigm, as there were

significant differences ($P<0.05$) in saline-treated



TS= T. Spathacea; DZ=Diazepam;n = 6 in each group. Values are expressed as Mean ⍰} SE. P values: ***<0.001; **<0.01;*<0.05 as compared to saline-treated
group. Statistical test employed was ANOVA followed by Student-Newman–Keul's post hoc test
Figure 1: Anxiolytic effects of *T. Spathacea* extract expressed by the percentage time spent in open arms (a) and number of open arm
entries (b)



TS= T. Spathacea; DZ=Diazepam;n = 6 in each group. Values are expressed as Mean ⍰} SE. P values: ***<0.001; **<0.01;*<0.05 as compared to saline-treated
group. Statistical test employed was ANOVA followed by Student-Newman–Keul's post hoc test
Figure 2: Effect of different treatments on the time spent by mice behavior in light and dark test
furthermore as plant extract treated groups (Figure 3a). There was decrease in locomotion activity in extracttreated (200 mg/kg) groups because the range as the number of squares crossed in the perimeter was decreased between extract-treated groups and differed significantly (P<0.05) from the control groups. The frequency of rearing also decreased significantly (P<0.001) after administration of hydroalcoholic extract of *T. spathacea* at 100 and 200 mg/kg compared to salinetreated group (Figure 3b).

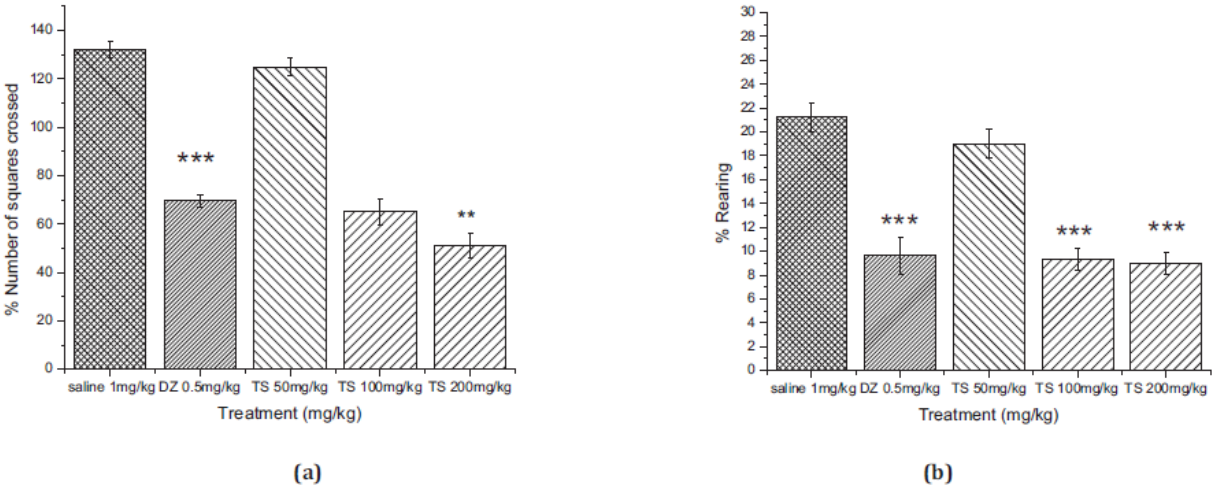
Social Interaction Test

No significant effects were produced at 50 mg/kg of the plant extract of *T. spathacea* in social interaction test. Diazepam (0.5 mg/kg) significantly (P<0.001) increased the time spent in social interaction among mice as compared to its effect in the saline-treated group (Figure 4). *T. spathacea* hydroalcoholic extract at 100 mg/kg (P<0.01) and 200 mg/kg (P<0.001) significantly

increased the time spent in social interaction as compared to salinetreated group (Figure 4). No significant effects were produced at 50 mg/kg dose of *T. spathacea* hydroalcoholic extract.

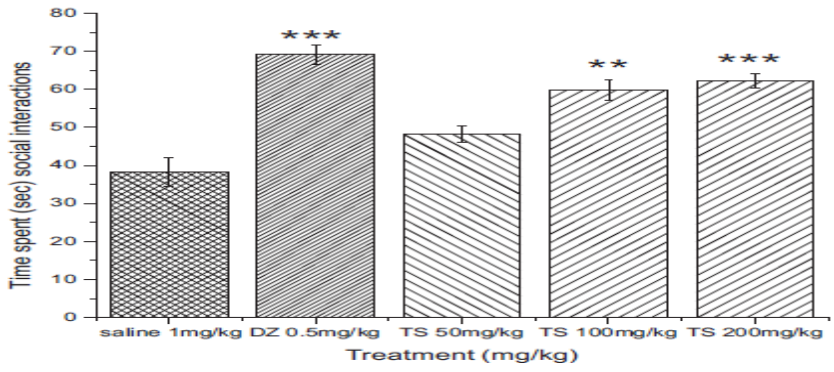
DISCUSSION

Benzodiazepines have been extensively used for the last few decades to treat several forms of anxiety, but due to their unwanted side effects, alternative treatment strategies with favorable side effect profiles, credible benefits and moderate costs are of interest, especially in primary care settings. Medicinal plants are a good source to find new remedies for these disorders. In the search for an alternative, more specific and perhaps cost-free therapy, research has been conducted to investigate natural anxiolytic drugs as well as new antidepressant principles. [23] The effects of 100 and 200 mg/kg of hydroalcoholic



TS= T. Spathacea; DZ=Diazepam;n = 6 in each group. Values are expressed as Mean ⍰} SE. P values: ***<0.001; **<0.01;*<0.05 as compared to salinetreated
group. Statistical test employed was ANOVA followed by Student-Newman–Keul's post hoc test

Figure 3: Effect of different treatments on the time spent by mice behavior in open field test



TS= T. Spathacea; DZ=Diazepam;n = 6 in each group. Values are expressed as Mean ⍰} SE. P values: ***<0.001; **<0.01;*<0.05 as compared to salinetreated
group. Statistical test employed was ANOVA followed by Student-Newman–Keul's post hoc test

Figure 4: Effect of different treatments on the time spent by mice behavior in social interaction test

extract of *T. spathacea* on the EPM, light dark test, OF test (200 mg/kg only) and social interaction test were almost equivalent to that of 0.5 mg/kg diazepam, showed that T. spathacea has an active anxiolytic activity at 100 mg/kg dose. In the present study, the anxiolytic activity of the *T.*

spathacea extract was observed at doses of 100 and 200 mg/kg in mice. These observations clearly indicate that *T. spathacea* exerts an anxiolytic activity. Anxiolytic activity of *T. spathacea* is likely to be associated with its essential oil content and flavonoids. Mechanism of action by which *T. spathacea* shows anxiolytic activity may be similar to that of diazepam, as flavonoids and diazepam are structurally similar. [24] Effects of flavonoids as anxiolytics have been observed in many plant species used as folk medicine, such as *T. spathacea*, also used for the management of CNS disorders. [25] *T. spathacea* may be a useful in the management of neurodegenerative diseases. In summary, the hydroalcoholic extract of *T. spathacea* has an anxiolytic activity. Further pharmacological and chemical investigations are required to elucidate the exact mechanism of action of this extract and to isolate the active principles responsible for such effects.

CONCLUSION

The findings in this study suggest that the *T. spathacea* possess anti-anxiety activity. The results have been

obtained carefully from the controlled experiments model with laboratory animals. The statistical validity of the findings has been proven and they provide a scientific foundation for the use of the biologically active ingredients of *T. spathacea* in anxiety for explain the clinical importance of the *T. spathacea*. Further studies would be necessary to evaluate the contribution of active chemical constituents for the observed anti-anxiety activity as it still remains to be determined which components were responsible for these effects.

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GST IMPACT ON PHARMA AND HEALTHCARE INDUSTRY IN INDIA

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ABSTRACT

Goods and Services Tax (GST) is an indirect tax levied in India on the sale of goods and services. Goods and services are divided into five tax slabs for collection of tax - 0%, 5%, 12%, 18% and 28%. The tax came into effect from July 1, 2017 through the implementation of One Hundred and First Amendment of the Constitution of India by the Modi government. The tax replaced existing multiple cascading taxes levied by the central and state governments. The tax rates, rules and regulations are governed by the Goods and Services Tax Council which comprises finance ministers of centre and all the states. GST simplified a slew of indirect taxes with a unified tax and is therefore expected to dramatically reshape the country's 2 trillion dollar economy.

Keywords: GST, Indirect tax, Unified tax

INTRODUCTION

GST is hailed as the biggest tax reform since independence. It will include all taxes at various stages of value addition in production process of goods and services i.e. buying raw material, manufacturing of components and final product, warehousing, and transportation and final sale to customer. These taxes were levied by multiple authorities such as local (municipalities), state and central governments. The final customer will pay GST while purchasing from the last dealer. Thus it is not a new tax but replaces all taxes which were levied at all the previous stages in production and sale process with one tax.

Now there is one tax with two components i.e. state components and central. The state component will go to the state in which final transaction took place and central component will go to central government. GST is expected to increase the government revenue as tax evasion will be checked and many services that were not under the service tax regime will come under GST. The increase in Government revenue will improve investment in health and the social determinants of health. It will also provide transparency and certainty in the Indian tax system. It will improve the ease of doing business in India for both local and off-shore investors. India’s current standing globally in ease of doing business is 130 out of 190 countries. Globally, GST is seen as a simple, efficient and successful form of indirect tax reform. It will contribute to accelerate economic growth in India by replacing the current multiple (more than 15), inefficient, irrational and complex indirect tax system in India.

GST and healthcare sector

Health care is one of the fastest growing sectors of the Indian economy with lots of potential in terms of revenue and employment. Health care is a wider term that mainly includes pharmacy, medical devices, medical insurance, diagnostics and other components of medical care. The GST is going to affect all the components of health care in various ways.

1) GST and Pharmaceutical industry

About two thirds of the out of pocket expenditure on healthcare is on drugs in India. The burden of all the taxes on drugs in general was about 13 percent in the pre GST period and the current GST is 12 percent as a whole including ayurvedic drugs. The medicines for HIV-AIDS, malaria, tuberculosis and diabetes will be imposed 5 percent GST. The GST on the drugs produced under excise free manufacturing zone is yet to be clarified. The best thing for the pharma companies is that their cost of purchase is going to reduce. Moreover the burden of multiple tax and complexities associated with multiple tax system slowed down the business. GST will give hassle free business environment to the pharma companies. For the consumer the cost of drugs will come down.

2) GST and Medical devices and Equipment

The manufacturers of medical devices are also joining the party as medical devices and surgical equipments are proposed to be taxed 12 percent under the GST. The previous burden of taxes on the medical devices and equipment was over 13 percent including all the bunch of taxes. So one percent tax benefit is clearly visible under the new tax system for the medical device and equipment industry. This will clearly give a boost to the industry in the near future. The consumer will also share the benefits in terms of lower price and affordability.

3) GST and Health Insurance

There is lot of scope of for health insurance in the country like India where the coverage under health insurance is only 18 percentage in urban and 14 percent in rural India in 2016. The GST rate on the insurance sector is 18 percent as against 15 percent service tax in the pre GST era. It clearly indicates that the health insurance premiums are going to increase.

4) GST and diagnostics

There is expected rise in the prices of diagnostics such as blood tests, X-rays, MRI and strip based diagnostics as they are put under either 12 or 18 percent slab which is higher than the previous tax rate on these services. In the pre GST era the 10-15 percent of out of pocket expenditure is on diagnostics which is expected to increase in the post GST period.

CATEGORY	PRE - GST	POST - GST
Pharmaceutical industry	13 %	12 %
Medical devices and Equipment	13 %	12 %
Health Insurance	18 %	15%
Diagnostics (blood tests, X-rays, MRI and strip based diagnostics)	10-15 %	12-18 %

CONCLUSION:

GST will certainly increase the Government revenue in the country with more transparency in the tax system that will further simplify the tax structure. The economy is expected to grow at a faster rate. Every sector of the economy would have its share in the growth of the economy including healthcare sector. In a broad spectrum, it is an analyzing phase for the healthcare sector to see the impact of GST. The experts of the healthcare sector are confident that the post GST period will bring the strategic change and will create a positive environment by minimizing the obstacles and complexities in the growth of healthcare sector and have a positive impact to bring down the cost of health.

HEPATOPROTECTIVE ACTIVITY OF *TALINUMPORTULACIFOLIUM* FORSK, EXTRACT AGAINST PARACETAMOL INDUCED HEPATIC DAMAGE IN RATS

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الجرذان في الكبد بالتلف المستحث الباراسيتامول من مستخلص ، الطفيلية الكربوهيدرات تكاثر نشاط

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[a]Department of Pharmacy، Jawaharlal Nehru technological university، Kakinada، Andhra Pradesh، India - 533 003 ، الهند ، براديش أندرا ، كاكينادا ، التكنولوجية نهرو لال جواهر جامعة ، الصيدلة قسم ، البحوث في باحث
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مختصرة نبذة

العلاجية لنتائجها كنتيجة الهندي الطب في تقليديًا (العائلة: Portulacaceae) *Talinumportulacifolium* Forsk استخدام تم *Talinumportulacifolium* forsk لـ الوقائي التأثير حول منهجية دراسة أي إجراء يتم لم .والسكري والسيلان الكبد لالتهاب الأمراض لعلاج *Talinumportulacifolium* forsk من الوقائية للفعالية ادعاءات تقديم يمكن لذلك .الكبدية الأمراض لعلاج الكبدية. *Talinumportulacifolium forsk* من الميثانولي المستخلص دور في التحقيق على الحالية الدراسة ركزت اختلال تنتج (METP). METP من جرعة مستوى عند 250mg / kg b.wt / day po و 500 mg / kg b.wt / day po المصل مستوى خفض طريق عن (P <0.05) كبير معنوي Aspartate amino transferase (AST) ، Alanine amino transferase (ALT) ، القلوي الفوسفاتيز (ALP) الكلي المصل والبيليروبين (SB) ، من كبير بشكل زادت أنها حين في .سيليمارين ، القياسي الدواء هذا مع للمقارنة قابلة MetP آثار كانت .الجرعة على تعتمد بطريقة الجلوتاثيون مستوى (GSH) في المستحثة الكبدية الإصابات - الباراسيتامول (PCM) ضد METP لـ المفيد الدور المرضية النسيجية الملاحظات أكدت كعوامل كبيرة إمكانات تمتلك Talinumportulacifoliumforsk من الميثانولي المستخلص أن إلى النتيجة وتشير .الجرذان كبد.

ABSTRACT

Talinumportulacifolium Forsk (family: Portulacaceae) has been traditionally used in Indian medicine as a result of its curative results of hepatitis, gonorrhea and diabetes. No systemic study has been done on protective effect of Talinumportulacifolium forsk to treat hepatic diseases. So claims can be made for the protective efficacy of Talinumportulacifoliumforsk to treat hepatic diseases. The present study focused on investigating the role of methanolic extract of Talinumportulacifolium forsk (METP). METP at a dose level of 250mg/kg b.wt/day p.o and 500mg/kg b.wt/day p.o produce significant ($P<0.05$) hepatoprotection by decreased the level of serum Aspartate amino transferase (AST), Alanine amino transferase (ALT), alkaline phosphatase (ALP) and Total serum bilirubin (SB), while they significantly increased the level of glutathione (GSH)in a dose dependent manner. The effects of METP were comparable to that standard drug, silymarin. Histopathological observations confirmed the beneficial role of METP against Paracetamol (PCM) - induced liver injury in rats. The result suggests that the methanolic extract of Talinumportulacifoliumforsk possess significant potential as hepatoprotective agents.

Key words: Hepatoprotective, Talinumportulacifolium forsk, Paracetamol, Lipid Peroxidation, Glutathione

Junapudi Sunil , Yasodha Krishna Janapati , Pallaval Veera Bramha; Hepatoprotective Activity of *talinumportulacifolium* forsk, extract against Paracetamol induced Hepatic damage in Rats;

1.INTRODUCTION

Talinumportulacifolium forsk is an annual herb belongs to the familyPortulacaceae[1] which mainly occurs in India, W. Peninsula, China and Ceylon. In India it is found in Andhra Pradesh and Tamil Nadu[1]. *T. Portulacifolium* forsk has been frequentlyused as an alternative, astringent to the bowels, worms, itching, useful in gonorrhea[1, 2]. The juice of the leaves of the plant is used for the treatment of diabetes, cures ulcers and traditionally used for the treatment of antioxidant[2-4].Since no scientific data are available to justify the traditional hepatoprotective potential of the plant.

Acetaminophen (N-acetyl-p-aminophenol, Paracetamol) is usually used as an analgesic and antipyretic drug[5]. Extensive use of PCM for therapeutic functions leads to severe hepatic damage. Toxic doses of PCM could induce changes in the morphology and function of liver mitochondria[6]. Formation of N-acetyl-p-benzoquinone imine (NAPQI) is the responsible for liver injury through depletion of glutathione (GSH) even as it binds to cellular proteins[7]. PCM induced hepatotoxicity is known to involve liver cytochrome P_{450} (CYPs) together CYP2E1, CYP3A4, and CYP1A2 and it also inhibits the mitochondrial oxidative phosphorylation, reduction of adenosine triphosphate (ATP) and produces selective mitochondrial oxidant stress[8]. Cellular necrosis of the liver cells raises the lipid peroxidation and depletion of glutathione (GSH) besides elevating the serum biochemical marker levels[5].

The survey of literature reveals that the *T.Portulacifolium* forsk are found to be used in the traditional system of medicine as a liver tonic[2]. However, hepatoprotective activity of *T.Portulacifolium* forsk has not been scientifically investigated. Therefore, in the present study hepatoprotective effect of methanolic extract of *T. Portulacifolium* forsk have been evaluated against paracetamol induced liver damage in the wistar albino rats.

2. MATERIALS AND METHODS

2**.1. Chemicals:**

Paracetamol 500 mg tablets (Nirmal Prime, Mumbai, India). Silymarin was purchased from Micro labs, Tamilnadu, India. Moreover, saline was purchased from GSN pharmaceutical private limited, Hyderabad, Telangana, India. The following biochemical parameters of AST, ALT, ALP and Bilirubin kits were obtained from Span Diagnostics, Surat, India. Rat's feed was once supplied from Mahaveer Endeavors, Medipally and Hyderabad, India. All other chemicals and reagents used in the study were of analytical grade.

2.2. Plant materials:

The plant of *T. portulacifolium* forsk was collected from mature plant during the month of November from the wood's territory of the Tirumala Hills, Tirupathi, Chitoor district. Andhra Pradesh (India). The plant material was taxonomically identified by Dr. K. MadhavaChetty, Department of Botany, Sri Venkateshwara University, Tirupathi, Andhra Pradesh, India and a specimen was kept in the herbarium (TP No; 2161). The plant materials were washed thoroughly to remove adhering soil and earthen matter, later on sliced into thin chips and dried in shade at room temperature and then after ground to optimal coarse powder.

2.3. Preparation of Extracts:

The powder (600 gm) was extracted at ambient temperature and 60^{o}C successively with (95% methanol). The solvent was changed at regular intervals of every 24 h. The alcohol from the pooled extractions were removed through distillation under reduced pressure at 50-60^{o}C to withstand METP (50.2g). The extracts were then subjected to preliminary phytochemical investigations and subjected forhepatoprotective activity against PCM- induced liver damage.

2.4. Preliminary Phytochemical Studies:

The extract of *T. portulacifolium* forsk were subjected to preliminary phytochemical screening for the detection of various phytochemical constituents such as carbohydrates, proteins, amino acids, steroids, tannins, flavonoids, terpenoids, alkaloids, mucilage and glycosides, using standard procedure[9,10], to find out the nature of phytoconstituents present within them

2.5. Experimental animals:

An experimental study was carried out using Wister albino rats of either sex (male and female) rat's age two months. Their body weights ranged from 150 to 200 g. The rats were divided into 5 groups of 6 (3 male and 3 female) animals per cage was used. Animals were maintained under standard laboratory aseptic conditions (12-h light/dark cycle, 24hrs). The food in the form of dry pellets and water is provided *ad libitum*. All the animals were accepted by the ethics approval committee of the institute. (Reg No: 1648/PO/A/12/CPCSEA).

2.6. Paracetamol (PCM) Induced Liver Toxicity:

The paracetamol (PCM) was diluted with saline (vehicle) prior to oral administration (o.p). The group I: vehicle (saline) once daily for 9 days. Group II: vehicle + PCM (1 mL/kg, p.o) once daily for ninth day. Group III: Silymarin (100 mg/kg b.wt/day, p.o) + PCM (1 mL/kg, p.o) once daily for ninth day. Group IV: AETP (250 mg/kg b.wt/day, p.o) + PCM (1 mL/kg, p.o) once daily for ninth day. Group V: AETP (500 mg/kg b.wt/day, p.o) + PCM (1 mL/kg, p.o) once daily for ninth day. To enhance the acute liver damage in animals of groups V, IV, III and II, food were withdrawn 12 h before PCM administration. Animals were sacrificed 24 h after administration of PCM. Blood samples were further collected and pooled by puncturing the retro-orbital plexus underneath using mild ether anesthesia and allowed to coagulate for 30 min at 37°C. Serum was isolated by centrifugation at 2500 rpm for 15 min at 35°C and further analyzed for various biochemical parameters [9-11].

2.7. Assessment of Liver Functions:

The hepatoprotective impact of extract was assessed by the measure of liver, biochemical markers. Alanine Amino Transferase (ALT)[14],Aspartate Amino Transferase (AST)[15], Alkaline Phosphatase

(ALP)[16] and Total Serum Bilirubin (SB)[13], Lipid Peroxidation (LPO) as Malondialdehyde (MDA)[17] and Glutathione (GSH)[18] according standard methods. Histopathological assessment of liver damage was done by studying Haematoxylin and Eosin (H&E) stained slides of liver tissue, including cell necrosis, fatty changes and lymphocytes[19-21].

2.8. Measurement of Antioxidant Activity:

From all the experimental groups, liver was collected and rinsed with 0.15 M Tris-HCl (pH 7.4). A 10% w/v of liver homogenate was prepared in 0.15 M Tris-HCl buffer and processed for biochemical estimation of lipid peroxidation in the form of malondialdehyde (MDA) in the liver[22].A part of homogenate after precipitating protein was used for estimation of reduced glutathione (GSH) [18]. The rest of the homogenate was centrifuged at 1500 rpm for 15 min at 4^0C.

2.9. Determination of lipid peroxidation in liver homogenate[22]

To 0.5 mL of homogenate tissue, 0.6 mL reagent (N-methyl -2-phenylindole and acetonitrile; 3:1) 1ml BHT (butylatedhydroxyltoluene) were added, mixed well and centrifuged at 3000 rpm at 10 min and boiled for 1h at 45^0C, the tubes were then cooled at room temperature and measured absorbance (UV-spectrophotometer, model UV-1601, Shimazu Corporation, Kyoto, Japan) at 586 nm.

3.0. Determination of reduced glutathione (GSH)[18]**:**

Homogenate tissue (0.2 mL) was mixed with 3.0 mL precipitating reagent (1.67g potassium phosphate, 0.2g EDTA, 30g P-nitro benzyl chloride (PNBS) in 1 L of distilled water) was added, mixed thoroughly and kept for 5 min before centrifugation. To 2.0 mL of the filtrate and absorbance measured at 310 nm.

3.1. Statistical Analysis:

The data was represented as mean ± SEM. Results were analyzed statistical by one way ANOVA test followed by Dunnet's assessment test using orgipro (Version 7.0). The minimum level of significance was set at $P<0.05$.

3. RESULTS

Preliminary phytochemical investigation revealed the presents of flavonoids, phenols, terpenoids and steroids in methanolic extract.

3.1. Paracetamol (PCM) Induced Liver Toxicity:

The results of hepatoprotective activity of METP on PCM treated rats are show in Table 1. The hepatic enzymes AST, ALT, ALP and SB in serum significantly ($P<0.001$) increased in PCM treated animals compared to normal control (group-I). The METP treatments (250 and 500mg/kg p.o) significantly ($P<0.05$, $P< 0.01$; respectively) the levels of hepatic enzymes when compared to PCM-treated animals. Silymarin (100mg/kg) - treated animals also show significant ($P<0.001$) the levels of hepatic enzymes when compared with to PCM-treated animals. There was significantly decreased ($P<0.001$) in the serum total serum albumin levels in PCM treated groups when as compared to the control groups, which was significantly ($P<0.001$) with treated of METP 500mg/kg b.wt/day and 250mg/kg b.wt/day, respectively.

Table 1: Effect of methanolic extraction of *T. Portulacifolium* forsk on ALT, AST, ALP and SB in PCM induced liver toxicity in rats

Treatment	Dose	ALT (U/L)	AST (U/L)	ALP (U/L)	SB (mg/dl)

Group-I Vehicle (saline)	1ml/kg	57±1.06	52±2.89	103.33±6.52	0.55±0.02
Group-II Control (PCM)	1 mg/kg	220.66±2.52[a]	190.33±3.02[a]	248.16±5.38[a]	2.03±0.17[a]
Group-III PCM+ Silymarin	100 mg/kg	172±1.75[***]	133.50±3.68[***]	198 .5±3.83[***]	0.93±0.07[***]
Group-IV PCM+ METP	250 mg/kg	209± 2.95[**]	160.33±2.58[**]	228.63±6.42[ns]	1.07±0.06[***]
Group-V PCM+ METP	500 mg/kg	191.83±6.17[***]	142.16±4.66[***]	212.16±6.73[*]	0.99±0.156[***]

*Each value represents the mean ± SEM. n =6 number of animals in each group. [a]P<0.001 vs vehicle control, *P<0.05, **P<0.01, *** P<0.001, Compared to respective PCM treated control groups*

3.2. Effect of *T. Portulacifolium* forsk on antioxidant activity:

There was a significant increase in MDA content and decrease in GSH activities of PCM intoxicated animals. Pre-treatment with silymarin (100 mg/kg b.wt/day) and *T. Portulacifolium* forsk (250 and 500 mg/kg p.o) significantly $P< 0.05$ prevented the increase in MDA levels and brought them near to normal level, whereas GSH levels were significantly ($P< 0.001$) raised, thus providing protection against paracetamol toxicities.Results are given Table 2.

Table 2: Effect ofmethanolic extraction of *T.Portulacifolium* forsk on lipid peroxidation (LPO), glutathione (GSH), PCM induced hepatic damage in rats

GROUP	DOSE	LPO (nM MDA/mg protein)	GSH (µg/mg protein)
Group-I Vehicle (saline)	1ml/kg	0.93±0.03	6.17±0.24
Group-II Control (PCM)	1ml/kg	4.18±0.18[a]	2.26±0.30[a]
Group-III PCM+ Silymarin	100 mg/kg	2.11±0.22[***]	5.01±0.10[***]
Group-IV PCM+ METP	250 mg/kg	2.21±0.11[***]	4.30±0.17[***]
Group-V PCM+ METP	500 mg/kg	2.55±0.23[***]	4.94±0.06[***]

*Each value represents the mean ± SEM. n =6 number of animals in each group. [a]P<0.001 vs vehicle control, *P<0.05, **P<0.01, *** P<0.001, Compared to respective PCM treated control groups*

3.3. Histopathological examination of rat livers:

On the ninth day, later the animals were sacrificed and liver tissues were gathered. In this study, histopathological observation of liver was performed to further support the biochemical analysis evidence. The model group revealed the most severe damage of all the groups; Microscopic view of liver tissue of silymarin and methanolic extracts of *T. Portulacifolium* forsk on ALT, AST, ALP

and SB in PCM induced liver toxicity in rats rat. However, histological changes in liver tissues from groups which treated at dose 250 and 500 mg/kg p.o. (Fig 1A- E).

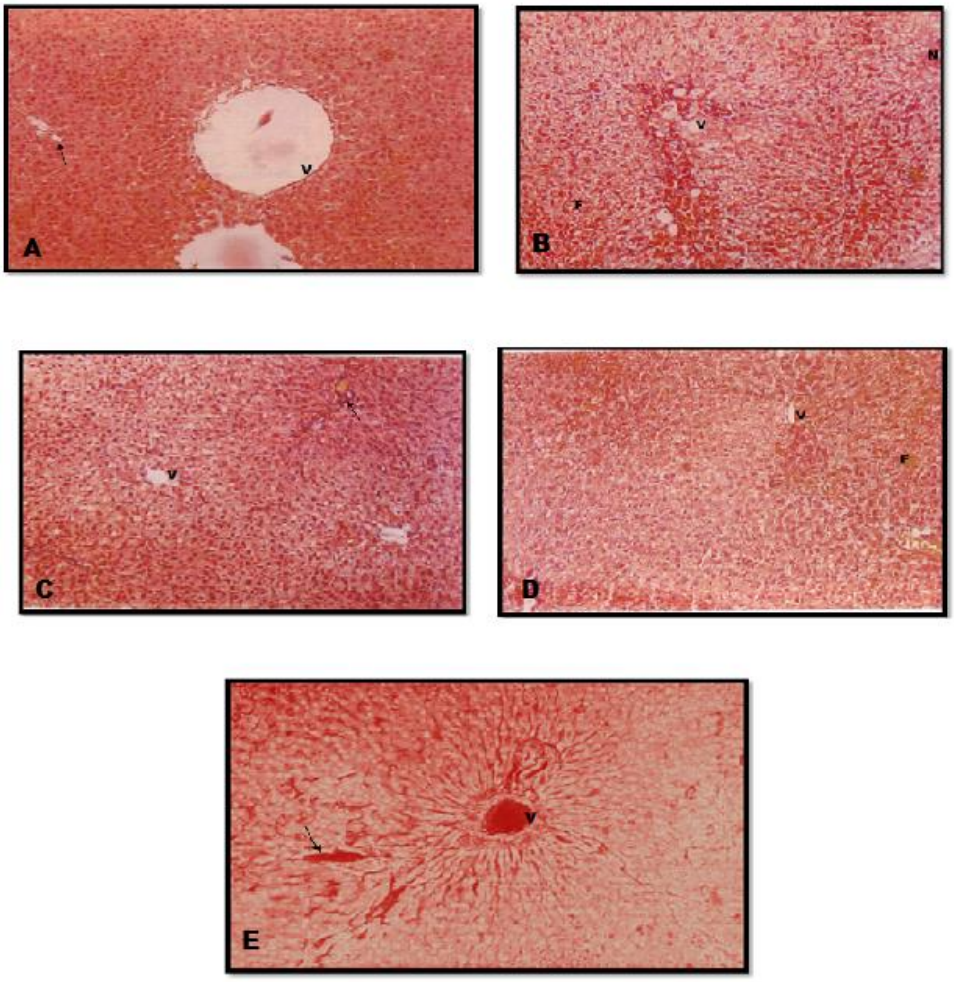

Figure 1:A) Microscopic view of liver tissue of normal rats; (B) Microscopic view of liver tissue of PCM; (C) Microscopic view of liver tissue of PCM + Silymarin; (D) Microscopic view of liver tissue of PCM + 250 mg/kg, po plant extract, (E) Microscopic view of liver tissue of PCM + 500 mg/kg, po plant extracts

Figure A: Liver tissues of control animal showing normal histology, section of normal liver tissue with portal triad showing portal vein (V), portal artery (arrow) and hepatic ducts (arrow head). Stain H and E, magnification 100X (Group I); Figure B: Liver tissue of animal treated with PCM showing necrosis, section of live tissue of animal treated with PCM showing necrosis (N), fatty vacuole (F) and central vein (v). Stain H and E, magnification 100x (Group II); Figure C: Liver tissue of PCM + Silymarin treated animals showing normal hepatocytes, section of normal liver tissue with portal triad showing portal vein (V), portal artery (arrow) and hepatic ducts (arrow head). Stain H and E, magnification 100X (Group III); Figure D: Liver tissue of PCM + 250 mg/kg b.wt, poMETP showing normal arrangement of hepatocytes, section of the liver tissue of PCM + 250 mg/kg b.wt, po METP treated animals showing normal arrangement of hepatocytes around the portal vein (V), absence of necrosis and moderate accumulation of fatty vacuoles (F). Stain H and E, magnification 100X(Group IV); Figure E: Liver tissue of PCM + 500 mg/kg b.wt, po CETPF showing normal arrangement of hepatocytes, Section of the liver tissue of PCM + 500 mg/kg b.wt,

poMETP treated animals showing normal arrangement of hepatocytes around the portal vein (V), portal artery (arrow) and hepatic ducts (arrow head). Stain H and E, magnification 100X(Group-V)

4. DISCUSSION

The *T. Portulacifolium* forsk extract has been reported to contain different types of terpenoids, the phytochemical screening. A number of compounds belonging to the class of polyphenol have been suggested to possess antioxidant and hepatoprotectiveactivitys[23]. Extensive liver damage by paracetamol itself decreases its rate of metabolism and other substrates for hepatic microsomal enzymes[24]. Interestingly the induction of cytochrome P_{450} or depletion of hepatic glutathione is a prerequisite for paracetamol-induced toxicity[25, 26].The hepatoprotective activity of *T. Portulacifolium* forsk(500 mg/kg, p.o and 250mg/kg p.o) was compared with the activity of standard silymarin (a hundred mg/kgb.wt/day). Pretreatment of animals with methanolic extracts of *T. Portulacifolium* forsk and silymarin prevented the Paracetamol induced rise in serum level of transaminases and total serum bilirubin, confirming the protective effects of methanolicextract of *T. Portulacifolium* forsk against Paracetamol induced hepatic damage.

However, there was no significant effect on rise in serum alkaline phosphatase levels by the test extract and silymarin. However the paracetamol induced liver necrosis was once inhibited significantly by using *T. portulacifolium* forsk extract, which confirms the protective action of methanolic extract of *T. Portulacifolium* forsk against experimentally induced liver damage in rats. ALT, AST, ALP and SB are the most sensitive tests employed in the diagnosis of hepatic disease. Therefore it can be concluded from this investigation that extract of *T. Portulacifolium* forsk possess hepatoprotective activity. Further, detailed studies are warranted to confirm the utility profile of this drug.

5.CONCLUSION

In conclusion, the result of this study demonstrates that *T. Portulacifolium* forsk has potent hepatoprotective action upon paracetamol-induced hepatic damage in rats. The present study thus justifies the traditional use of *T. Portulacifolium* forsk in treatment of liver diseases and also points out that *T. Portulacifolium* forsk warrants future detailed investigation as a promising hepatoprotective agent. However, the exact mechanism(s) and the active compound(s) involved in these effects need to be clarified in future studies.

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Ethical Approval

All authors hereby declare that "Principles of laboratory animal care" were followed, as well as specific national laws where applicable. All experiments have been examined and accepted by the institutional animal ethical Committee (Reg No: 1648/PO/A/12/CPCSEA). All authors hereby declare that all experiments were examined and approved by the institutional ethics committee and been performed in accordance with the ethical standards.

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Method Development and Validation for Simultaneous Estimation of Raltegravir and Lamivudine by Using RP-HPLC in Bulk and Pharmaceutical Dosage Form

Sunil Junapudi1*, P Nagaraju1, K Ganesh1, M Nagesh1

ABSTRACT

High performance liquid chromatography is at present one of the classiest tool of the analysis. The estimation of raltegravir and lamivudine was done by RP-HPLC. The Phosphate buffer was pH 3.0 and the mobile phase was optimized withconsists of acetonitrile: phosphate buffer mixed in the ratio of 45:55 % v/ v. Inertsil ODS 3V C18 column (4.6 x 150 mm, 5 _m) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out by using PDA detector at 275 nm. The solutions were chromatographed at a constant flow rate of 1.0 ml/min. the linearity range of raltegravir and lamivudine were found to be from 150-450 _g/ml of raltegravir and 50-150 _g/ml of lamivudine. Linear regression coefficient was not more than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 100.36 and 100.30% of raltegravir and lamivudine. LOD and LOQ were found to be within limit.

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INTRODUCTION

Raltegravir (RAL) is chemically N-[(4-Fluorophenyl)methyl]-1, 6- dihydro-5-hydroxy-1-methyl-2[1-methyl-1-
[[(5- methyl-1, 3, 4-oxadiazol-2-yl) carbonyl] amino] ethyl]-6-oxo-4 pyrimidine carboxamide. It is a human
immunodeficiency virus (HIV) integrase strand transferinhibitor. [1, 2] The chemical structure of RAL was shown infigure-1. Researchers found proof that few analyticalmethods such as UV, [3-6] HPLC, [7-12] UPLC, [13] LC-MS [14-15]and HPTLC [12] methods have been reported in either aloneor combined dosage form and biological sample

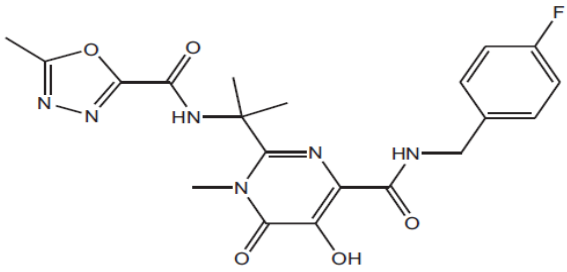

Figure 1: Chemical structure of raltegravir

Lamivudine (LAM) is chemically 4-amino-1-[(2R, 5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one. It is an HIV-1 nucleoside analogue reversetranscriptase and HBV polymerase inhibitor. [1, 16] Thechemical structure of RAL was shown in Figure 2.Researchers reveals that very few analytical methods havebeen reported for the determination of LAM which includesUV, [17-22] HPLC, [17, 23-27] HPTLC [17, 28] and LC-MS [29, 30] areavailable for the estimation of LAM either individually orcombined dosage form and biological sample.Correspondingly, this manuscript described theoptimization of an isocratic RP-HPLC method for the routine quality control analysis of LAM and RAL inlaboratory prepared binary mixture. In spite of thatDevelopment and optimization of isocratic RP-HPLCmethod is a tedious

process that involves instantaneousdetermination of several factors. [31-36] It is recognized toprovide risk-based understanding of the analytical as wellas major factors affecting the performance of analyticalmethod. [37-39] Furthermore, it provided thoroughunderstanding of the possible risk and associated withinteraction among the method variables, respectively. [40]Therefore, the aim of present study was todevelop,optimize and validate sensitive and cost-effective RP-HPLCmethod for estimation of LAM and RAL in laboratoryprepared binary mixtures.

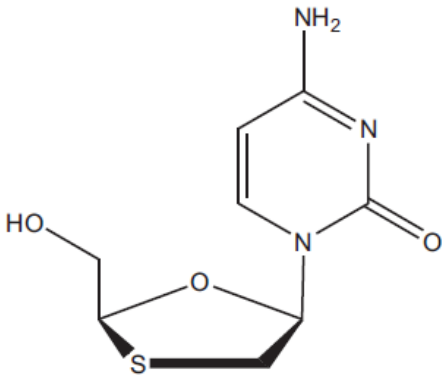

Figure 2: Chemical structure of lamivudine

MATERIALS AND METHODS

Chemicals

Pure drugs LAM (99.95%) and RAL (99.95%) were kindly supplied by Richer Pharmaceuticals, Prasanthinagar, Hyderabad, India and Emcure Pharmaceuticals, Pune, India respectively. The Pharmaceuticals LAM and RAL (DUTREBIS, Tablets: 150 mg lamivudine and 325.8 mg raltegravir potassium) were purchased from local pharmacy (Meda Pharmaceuticals/ Hyderabad, India). Dipotassium hydrogen phosphates (AR Grade), Ortho phosphoric acid (AR Grade), acetonitrile (HPLC Grade) were purchased from E. Merck (India) Ltd. Worli, Mumbai, India. The 0.45 µm nylon filters were purchased from Advanced Micro Devices Pvt. Ltd., Chandigad, India.

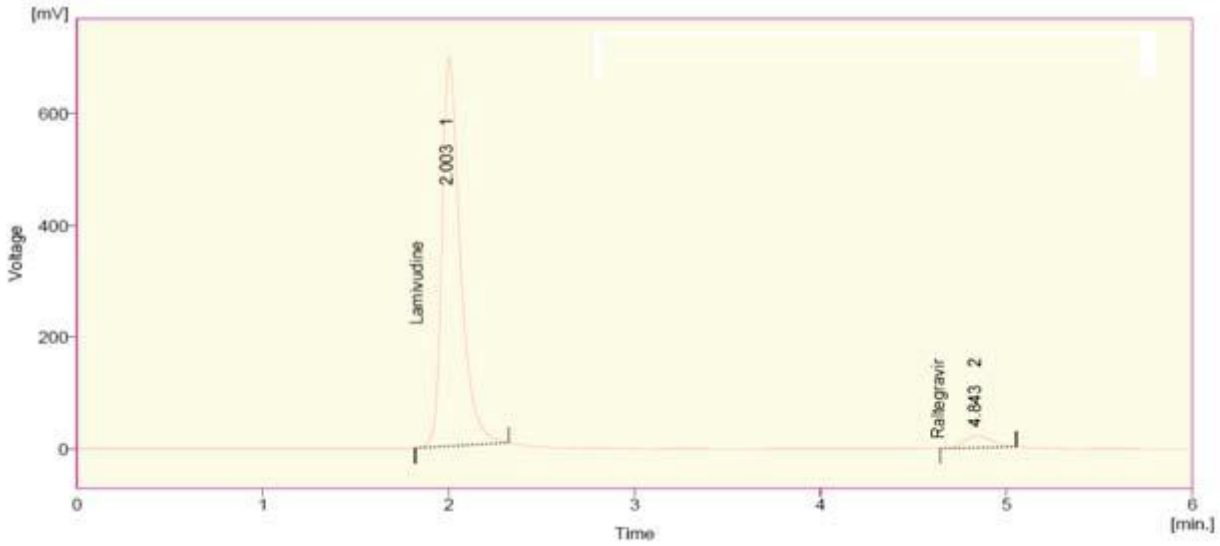

Figure 3: A typical chromatogram of a tablet sample solution containing RAL and LAM

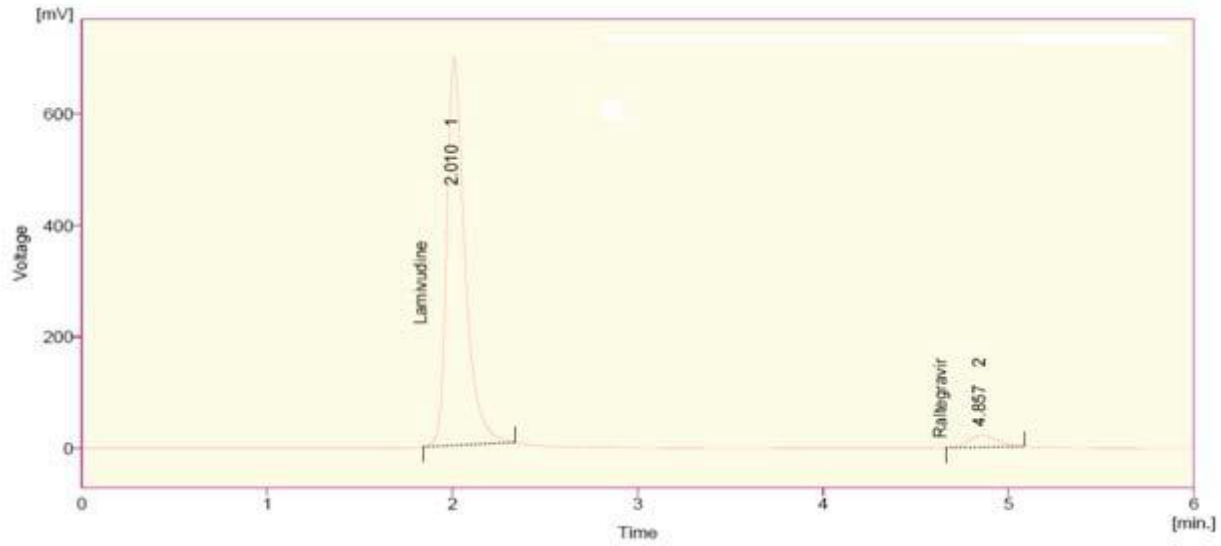

Figure 4: A typical chromatogram of Standard solution containing RAL and LAM

Equipments

Analysis was performed on a chromatographic system of Shimadzu (LC 20 AT VP) equipped with an auto injector, chromatographic system software was Spin chrome (LC SOLUTIONS). A chromatographic separation was achieved on Inertsil ODS 3V C18 column (4.6 x 150 mm, 5 _m) analytical column. Data acquisition was made with empower software.

Standard Solutions and Calibration Graphs

Standard stock solution of RAL (10.0 mg/ml) and LAM (5.0 mg/ml) was prepared in diluent which was a mixture of potassium dihydrogen phosphate with buffer (pH 3.0) and acetonitrile in proportion (55:45, v/v). To study the linearity range of each component, serial dilutions were made by adding this standard stock solution in the different weights of RAL in the range of 150–450 µg/ml of RAL and 50-150 µg/ml of LAM. A graph was plotted as concentration of drugs versus peak area response. It was found to be linear for both the analytes. From the standard stock solution, a mixed standard solution was prepared containing 10 µg/ml of RAL and LAM. The system suitability test was performed from five replicate injections of mixed standard solution.

Preparation of Buffer

1.625 grams of potassium Di hydrogen phosphate (KH2PO4) and 0.3 grams of dipotassium hydrogen phosphate was weighed and dissolved in 100 ml of water and volume was made up to 550 ml with milli-Q water. Adjust the pH to 6.5 • }0.05 using orthophosphoric acid. The buffer was filtered through 0.45µ filters to remove all fine particles and gases.

Preparation of Mobile Phase

A mixture of 450 ml of acetonitrile and 550 ml of buffer (45:55v/v) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45µ filter under vacuum filtration.

Diluents Preparation

The Mobile phase was used as the diluent.

Sample Preparation

Twenty tablets (Each tablet contains RAL-300 mg and LAM-150 mg) were weighed and finely powdered. The average weight of tablets is determined with the help of weight of 20 tablets. A portion of powder equivalent to the weight of one tablet was accurately weighed into 100 ml Agrade volumetric flasks and 50 ml diluents were added. The volumetric flasks were sonicated for 20 min to effect complete dissolution of the RAL and LAM; the solutions were then made up to volume with diluent. The solution was filtered through 0.45 µm nylon filter. The aliquot portion of the filtrate was further diluted to get final concentration of 15 µg/ml of LAM and 30 µg/ml of RAL.

Ten microlitres of the test solution was injected and chromatogram was recorded for the same and the amounts of the drugs were calculated.

Method Validation

The HPLC method was validated in terms of precision, accuracy and linearity according to ICH guidelines. [32, 41, 42] Assay method precision was determined using nineindependent test solutions. The intermediate precision of the assay method was also evaluated using different analyst on three different days. The accuracy of the assay.

Table 1: Results of the Recovery Analysis of RAL and LAM

Compound	%Concentration (at Specification Level)	Area	Amount Added (µg)	Amount Found (µg)	Recovery %	Mean Recovery
RAL	50%	2713697	150	150.89	100.68%	
	100%	2058547	300	298.65	96.36 %	100.36 %
	150%	325034	450	453.76	104.04 %	
LAM	50%	5649981	75	75.98	101.59%	
	100%	4721.589	100	100.01	100.01%	100.30 %
	150%	6582420	150	148.98	99.32%	

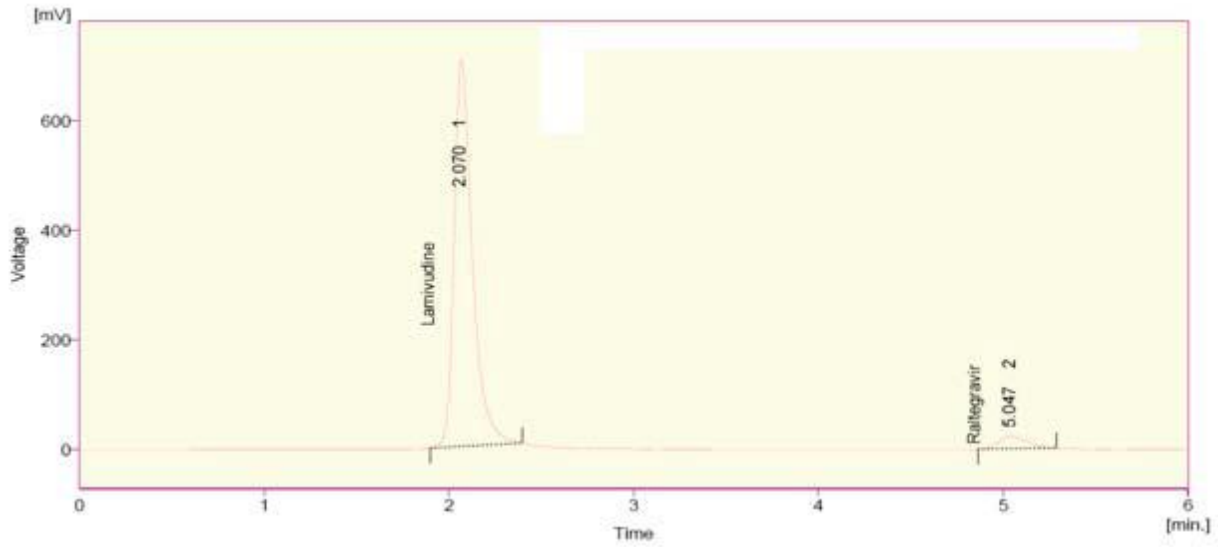

Figure 5: A typical chromatogram of tablet sample solution containing RAL and LAM

method was evaluated with the recovery of the standards from excipients. Three different quantities (low, medium and high) of the authentic standards were added to the placebo. The mixtures were extracted as described in above and were analyzed using the developed HPLC method. Linearity test solutions were prepared as described above.The LOD and LOQ for analytes were estimated by injecting a series of dilute solutions with known concentration. To determine the robustness of the method, the final experimental conditions were purposely altered and the results were examined. The flow rate was varied by (• }) 0.1ml/min. The percentage of organic modifier was varied by (• }) 5% and pH of mobile phase was varied by (• }) 0.1.

RESULTS AND DISCUSSION

Optimization of the Chromatographic Conditions

During the analysis of basic drugs like RAL and LAM, one of the well known problems in pharmaceutical industry is peak tailing. Since these compounds strongly interact with polar ends of HPLC column packing materials, causing severe peak asymmetry and low separation efficiencies. High purity silica backbone and advances in bonding technology have alleviated the tailing problem of polar compounds in HPLC to a significant extent. During the optimization of the method, different columns (Inertsil C18 150 mm×4.6 mm, 5_m; Zorbax C18 250 mm×4.6 mm, 5 µm; Symmetry C18 250 mm×4.6 mm, 5 µm) and two organic solvents (acetonitrile and methanol)

were tested. The chromatographic conditions were also optimized by using different buffers like phosphate, acetate and citrate for mobile phase preparation. After a series of screening experiments, it was concluded that mixer of phosphate buffers gave better peak shapes than their acetate and citrate counter parts. With methanol as solvent both the peaks shows less theoretical plates and more retention time compared to acetonitrile. The chromatographic separation was achieved on a Inertsil ODS C18 150 mm×4.6 mm, 5 _m column, by using a mixture of buffer and acetonitrile in proportion (55:45, v/v) as mobile phase, the detection was carried by using PDA detector. At Ambient column temperature and pH 3.0 of mobile phase, the peak shape RAL and LAM was found symmetrical. The flow rate kept was 1 ml/min and wavelength 275 nm to achieve adequate retention time of two peaks (Figure 3, 4).

Validation of Method

1. Accuracy

Accuracy of the method was calculated by recovery studies at three levels by standard addition method (Table 1). The mean percentage recoveries obtained RAL and LAM were 100.36% and 100.30%, respectively.

2. Specificity

The specificity of the HPLC method is illustrated in Figure 5 where complete separation of dasatinib and lenvatinib was noticed in presence of tablet excipients. In addition there was no any interference at the retention time of dasatinib and lenvatinib in the chromatogram of placebo solution. In peak purity analysis with photo diode detector, purity angle was less than purity threshold for both the analytes. This shows that the peak of analytes was pure and excipients in the formulation did not interfere the analytes.

3. Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The system precision is a measure of the method variability that can be expected for a given analyst performing the analysis and was determined by performing five replicate analyses of the same working solution. The relative standard deviation (R.S.D.) obtained for RAL and LAM was 0.94 and 1.11%, respectively.

4. Linearity

Linearity was determined for RAL in the range of 150µg/ml to 450 µg/ml and LAM in the range of 50 µg/ml to 150 µg/ml. The correlation coefficient (‘*r*’) values for both the drugs were >0.999. Typically, the regression equation for the calibration curve was found to be y=0.554x+66.39 for RAL and y = 35.94x+1185 for LAM.

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

LOD and LOQ of RAL and LAM were determined by calibration curve method. [41, 42] Solutions of both RAL and

LAM were prepared in the range of 0.45-300 µg/ml respectively and injected in triplicate. Average peak area of three analyses was plotted against concentration.

LOD and LOQ were calculated by using following Equations:

$$LOD = 3.3 \times Syx/b; \quad LOQ = 10.0 \times Syx/b$$

Where, Syx is residual variance due to regression;

b isslope. LOD and LOQ for RAL were 28.11 and 85.19 µg/ml respectively and for LAM were 0.43 and 1.31 µg/ml,respectively.

CONCLUSION

A simple, specific, linear, precise and accurate RP-HPLC method has been developed and validated for quantitative determination of RAL and LAM in new tablet formulation. The method is very simple and specific as both peaks are well separated from its impurities and excipient peaks with total runtime of 10min, which makes it especially suitable for routine quality control analysis work.

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HENOCH-SCHONLIEN PURPURA ASSOCIATED WITH HEPATITIS C AND COMPENSATED CIRRHOSIS

SAGAR PAMU[1*], RAMESH SARANGI[2], MOHAMMED ABUBAKAR[3]

ABSTRACT

An unordinary case report of henoch-schonlein purpura (HSP) in a relationship with hepatitis C and compensated cirrhosis was identified in 14 y aged male patient. He was admitted in the pediatric department with stomach pain, yellow skin, rashes with tingling and erythematous injuries over the legs with agony and swelling since multi-day. He feels pain during walking and appears to be with swelling of lower leg muscles. His unusual liver function test was distinguished with elevated levels of bilirubin-3 mg/dl, basic phosphatase-314 U/l, aspartate aminotransferase-55 U/l and alanine aminotransferase-60 U/l. His skin biopsy shows up leukocytoclastic vasculitis and IgA depositions. Liver biopsy revealed nuclei enlargement with extensive cell change and scattered cell plates. His blood test was with the presence of the hepatitis C virus (HCV) antibodies. He was finally diagnosed as HSP associated with HCV and compensated cirrhosis.

Keywords: Henoch-schonlein purpura, Hepatitis C, Immunoglobulin A, Compensated cirrhosis, Erythematous lesions

INTRODUCTION

HSP portrayed by generalized vasculitis [1]. HSP is characterized by non-thrombocytopenic palpable purpura, arthritis or arthralgias, gastrointestinal and renal involvement [2]. Inflammation also involves the blood vessels of the skin, lungs and focal sensory system with a mind-boggling store of immunoglobulin A (IgA) [3, 4] influencing transcendently in children [5]. HSP builds up the indications of rash, particularly finished legs, stomach pain, subcutaneous oedema, joint pain and glomerulonephritis [6]. HSP can influence any organ, physical examination observations are required. An exact reason for the cause of HSP is not known, but it is believed to be multifactorial, with hereditary, natural, and antigenic parts [7, 8]. It is difficult to distinguish by skin biopsy, which can show leukocytoclastic vasculitis and IgA depositions [9].

HCV is a major cause of liver disease and the potential cause of substantial morbidity and mortality worldwide [10]. Hepatitis C caused by HCV can be analyzed by the discovery of immunizing to HCV in the blood test by the immunoassay method [11] and identification of IgM antibodies against hepatitis C infection antigens [12]. HCV disease can do significant damage to the liver and leads to cirrhosis. It's not surprising that numerous individuals with hepatitis C don't know they have a life-threatening disease which can lead to liver cirrhosis. There are two phases of cirrhosis 1. **Compensated cirrhosis** means the body still functions despite decreased liver function and scarring. 2. **Decompensated cirrhosis** implies that body functions are separated where serious side effects like kidney failure, variceal haemorrhage, and hepatic encephalopathy may occur. Indications of cirrhosis are because of hepatitis C are fatigue, nausea, loss of appetite, weight reduction, wounding, itchy skin, jaundice, swelling in legs, ascites, hepatorenal disorder [13].

A few studies reveal hepatitis C associated IgA/IgM mixed cryoglobulinaemia can't be ruled out regardless of a negative cryoglobulin screen [14] on two occasions. In this patient, an IgA mediated vasculitis may have been the nidus for thrombus development and abdominal catastrophe. The role of liver cirrhosis in the advancement of HSP is fascinating. Patients may create HSP as because of an impact of abnormal liver metabolism of IgA circulating immune complexes that is an impaired clearance of IgA complex in

liver cirrhosis resulting about tissue depositions [15, 16], despite the fact that this is known to occur without overt vasculitis [16]. This mechanism appears to be in this case. The preceding HCV might have contributed to the formation of the immune complex.

There is no particular treatment for HSP. Acetaminophen or NSAIDs can be used for pain management and some of the time corticosteroids may also be used [17].

CASE REPORT

A male patient, aged 14 y admitted in the pediatric department with chief complaints of the stomach pain, yellow skin, rashes with tingling and erythematous sores over the legs with pain and swelling since one day. He feels pain during walking and appears to be swelling of lower leg muscles. There is no history of rash after food consumption, medicine use, insect bite, and there is no cough and swelling of the neck to the patient. His birth and improvement history was observed to be normal. Physical examinations, biochemical reports, complete blood picture and urine examinations seem to be normal. Peripheral smear test and ASO tube test were negative. His liver function test was observed to be elevated levels of total bilirubin-3 mg/dl, alkaline phosphatase-314 U/l, aspartate aminotransferase-55 U/l and alanine aminotransferase-60 U/l. His skin biopsy exhibits leukocytoclastic vasculitis with IgA depositions. Liver biopsy reveals nuclei enlargement with large cell change and disorganized cell plates. His blood test was positive with the presence of antibodies to HCV. He was finally diagnosed as HSP to have hepatitis C and compensated cirrhosis.

The patient was treated with IV ceftriaxone 1.125g+tazobactam 500 mg, IV netilmicin 50 mg BD, IV ranitidine 50 mg BD, IV pheniramine 1.5 cc BD, IV dexamethasone 1cc BD, tablet paracetamol 500 mg, tablet calcium, syrup multivitamin. He was counselled with a way of lifestyle changes in diet aspects.

DISCUSSION

An uncommon case report HSP association with hepatitis C and compensated liver cirrhosis were identified in a pediatric department. A male patient aged 14 y admitted in the pediatric department with stomach pain, yellow skin, rashes with rashes and erythematous injuries over the legs with pain and swelling since one day. He feels pain during walking and appears to be swelling of lower leg muscles. An elevated level of bilirubin, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase reveal liver damage to the patient. Patient's skin biopsy shows leukocytoclastic blood vessel vasculitis with IgA depositions, and liver biopsy reports with nuclei enlargement with a vast cell change of liver tissue which is considered as HSP Immunoassay method for a blood test of patient reports the presence of antibody to HCV indicates the condition of hepatitis C. The patient might be infected with HCV long back without creating any side effects. So it turned into a serious approach towards cirrhosis. There might be a blockage of flow in the biliary tract or buildup of biliary pressure in the liver. The patient's body functions well, although after understanding cirrhosis, so it was considered as compensated liver cirrhosis. A literature report says hepatitis C is associated with an IgA/IgM mixed cryoglobulinemia, but an IgA mediated vasculitis may have been the nidus for thrombus formation and abdominal catastrophe. The patient was developed HSP as due to the consequence of liver metabolism of IgA circulating immune complexes that is an impaired clearance of IgA complex in liver cirrhosis resulting in tissue deposition. This mechanism seems to be considered well for this patient. The preceding HCV might be formed in the patient due to the formation of immune. Nephritis is the most vital long-term prognostic factor in HSP; the transient prognostic factor is gastrointestinal illness can lead to death if there is no early treatment and care. He was treated with acetaminophen, corticosteroids, cephalosporin anti-infective agents and antihistamines. The way of lifestyle changes improves a

state of the liver. This case is considered to a remarkable case report because HSP in the early adult stage is uncommon; however, HSP usually occurs in the children.

CONCLUSION

An unordinary case report HSP association with hepatitis C and compensated cirrhosis require a nearby observing. Early conclusion and early treatment of hepatitis C are more essential it might prompt cirrhosis. A rate of HSP with cirrhosis has been expanding around the world. This condition has a long-term prognostic factor in causing nephritis, short-term factor to cause gastrointestinal disease in which both can lead to death if untreated, so early therapeutic intervention with clinical implications are needed.

ACKNOWLEDGMENT

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AUTHORS CONTRIBUTIONS

Sagar Pamu identified the case report and analyzed uncommon points; Sarangi Ramesh helped in editing this article and Mohammed Abubakar contributed the sources to analyze this case report.

CONFLICT OF INTERESTS

Author has no potential conflicts of interest with respect to the authorship and publication of this article.

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Geethanjali College of Pharmacy

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Vision

To be a Premier Pharmaceutical Education and Research Institution.

Mission

- Provide state of the art laboratories, information centre and learning environment for holistic education.
- Adopt and implement best practices for learning and research.
- Collaborate with industry and society to identify problems, provide sustainable solutions and align curriculum.

Program Educational Objectives (PEOs)

- PEO-1- KNOWLEDGE: Pharmacy Graduates will have professional & technical career in inter disciplinary domains providing innovative and eco-friendly sustainable solutions using modern tools.
- PEO-2- SKILLS: pharmacy graduates will have effective communication, leadership, team building, problem solving, decision making and creative skills.
- PEO-3- ATTITUDE: Pharmacy Graduates will practice ethical responsibilities towards their peers, employers, society and world at large.