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Prednisolone Loaded Tamarind Gum Microspheres for Colonic Delivery

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

The aim of this work was to formulate a novel multiparticulate system having pH sensitive property and specific enzyme biodegradability for colon specific drug delivery of Prednisolone (PD). Natural polysaccharide, Tamarind gum is used for microsphere preparation and Eudratit S- 100 for coating to provide pH controlled drug release. The formulation aims at minimal degradation and optimum delivery of the drug with relatively higher local concentration, which may provide more effective therapy for inflammatory bowel disease including Crohn disease and ulcerative colitis. Tamarind gum microspheres were prepared by emulsion dehydration technique using polymer in ratio of 1:1 to 1: 9. These microspheres were coated with Eudragit S-100 by oil in oil solvent evaporation method using core: coat ration (5:1). Tamarind gum microspheres and Eudragit coated tamarind gum microspheres were evaluated for surface morphology, particle size and size distribution, percentage drug entrapment, surface accumulation studies, in vitro drug release in simulated gastrointestinal fluids. The effect of various formulation variables were studied the prepared microspheres were spherical in shape in the size range of 64 µm to 113 µm, the encapsulation efficiency was in range of 30-72% depending upon the concentration of gum. The drug release was about 14-20% in first four hours of study gradually rises in 5th hour and 85% drug release occurs in 10-12% hr thus showing desirable drug release in the colonic simulated environment. PD tamarind gum microspheres are thought to have the potential to maintain drug concentration within target ranges for a long time, decreasing the side effects caused by concentration fluctuation, ensuring

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the efficiency of treatment and improving patient compliance by reducing dosing frequency. The animal study done using acetic acid induced colitis model on rats also suggest the anti inflammatory activity of the formulation.

Keywords: Inflammatory bowel disease; prednisolone; colon; microspheres; Eudragit Tamarind Gum.

1. INTRODUCTION

Inflammatory bowel disease (IBD) is a relapsing, debilitating, chronic, inflammatory disorder of the gastrointestinal tract (GIT), having two major forms: ulcerative colitis (UC) and Crohn's disease (CD). The difference between UC and CD is the fact that in UC, the inflammation occurs in the colon, while CD could affect any part of the GIT, commonly the terminal ileum or the perianal region [1]. Colon delivery of a therapeutic agent could reduce the systemic side effects and provide effective and safe therapy that may reduce the dose and duration of therapy when compared with the conventional treatment.

Presently various strategies have been used for targeting colon, like use of pH-sensitive polymers, coating with biodegradable polymers, synthesis of pro-drugs, timed release systems, embedding in biodegradable matrices and hydrogel system [2,3]. Polysaccharides, such as pectin, guar gum, chitosan and amylose, have extensively been studied and widely accepted for colon targeting [4]. The matrices of polysaccharides are used to remain intact in the physiological environment of stomach and small intestine, but after reaching the colon, they are acted upon by bacterial polysaccharases enzyme [5]. The combination of resistant core containing the drug coated with the pH sensitive polymer could result in better control over drug release from formulation. Also microspheres perform better in vivo than single unit systems as they spread thorough out the length of intestine causing less irritation, enjoy slower transit through the colon and give more reproducible drug release [6]. Tamarind gum matrices have been examined and tested for controlled drug delivery. Tamarind gum is degraded in colon by the enzymes released from colonic microbial flora and hence it can serve as an effective polysaccharide for colon-specific drug delivery [7]. Eudragit S 100 coating will provide pH sensitivity to the formulation.

2. MATERIALS AND METHODS

Prednisolone was obtained as gift sample from Kremoint Pharma Pvt Ltd Mumbai. Tamarind gum was obtained from Mangalwedhe tamarind industry, Bijapur, Eudragit S-100 (Research Lab), Span 80 (Merck India), n Hexane (Merck India), Ethanol (Research Lab), Acetone (Research Lab, Coconut oil (Research Lab) etc where of analytical grade.

2.1 Drug Excipient Compatibility Study

Drug excipient compatibility study was done by performing FTIR spectra of pure drug and Drug with polymers. Shimadzu made IR Tracer 100 model was used.

2.2 Preparation of Microspheres

The Tamarind Gum microspheres were prepared by emulsion dehydration technique. The drug-polymer dispersion was prepared in distilled water (20 ml). This dispersion was added in 50 ml coconut oil containing span 85 (1.25%w/v) and dispersion was continuously stirred at varied 400-800 rpm speed to obtain stable w/o emulsion. The emulsion is rapidly cooled at 15^{o}C Then 50 ml of acetone was added, in order to dehydrate the tamarind gum droplets. The system was maintained under mechanical agitation with propeller stirrer at 1000 rpm at room temperature for 20-40 min complete solvent evaporation. Microspheres were dried and washed with acetone, dried and stored in air tight container. Similarly, the tamarind gum microspheres with varying com-positions were prepared as shown in Table [8].

2.3 Coating of Microspheres

Tamarind gum microspheres were coated with Eudragit S-100 using oil-in-oil solvent evaporation method. Tamarind gum microspheres (50 mg) were dispersed in 10 ml of organic solvents mixture (acetone: ethanol, 2:1) containing Eudragit S-100 to give 1:5 core: coat ratio. This organic phase was added into 70 ml of light liquid paraffin containing 1% w/v span 85. The system was continuously agitated for 3 hr at 1000 rpm to evaporate the solvent at room temperature. Finally, the coated microspheres were filtered, washed with n-hexane and dried, stored in tightly capped container [9].

Table 1. Formulation of microspheres

Sr. No	Formulation Code	Drug : Tamarind Gum Ratio	Formulation Code	Core : Coat Ratio
1	T 1	1 : 1	ET 1	1 :5
2	T 2	1 :2	ET 2	1 :5
3	T 3	1 :3	ET 3	1 :5
4	T 4	1 :4	ET 4	1 :5
5	T 5	1 :5	ET 5	1 :5
6	T 6	1 :6	ET 6	1 :5
7	T 7	1 : 7	ET 7	1 :5
8	T 8	1 : 8	ET 8	1 :5
9	T 9	1 : 9	ET 9	1 :5

2.4 Evaluation of Microspheres

Scanning Electron Microscopy: The shape and surface morphology of tamarind gum microspheres and Eudragit- coated microspheres were investigated using scanning electron microscopy (SEM) [10] The dried microspheres were coated with gold foil (100Aº) under an argon atmosphere in a gold coating unit and scanning electron microscopy in both higher and lower resolution were observed. (Scanning Electron Microscope coupled EDAX model-JEOL-SEM- 6360).

Particle Size Distribution: The particle size distribution was done by the optical microscopy using a calibrated stage micrometer around 100 particles were calculated and mean diameter was calculated [11] The effect of polymer concentration on size was studied.

Percentage Yield: The percentage yield of all the batches were calculated on dry weight basis with respect to the solid materials added at the initial stage was calculated by using the following equation [12].

Percentage Yield = Amt of microspheres ***/*** Theoretical amount X 10

Drug Entrapment Efficiency: The drug entrapment efficiency was determined by dissolving 500 mg of microspheres in 100 ml of 0.2 M phosphate buffer pH 7.2 under sonication. After 24 hrs the filtrate was assayed spectrophotometrically at 246 nm. The drug content in the sample were calculated from the calibration plot and drug entrapment efficiency was calculated [13].

% Drug Entrapment = Calculated drug concentration/ Theoretical drug concentration× 100

2.5 *In vitro* Drug Release Studies

In vitro drug release studies were carried out using US Pharmacopoeia paddle type-II dissolution apparatus at 37 $\pm$ 0.5ºC with constant stirring rate of 100 rpm. 100 mg of Microspheres were used for the test. An accurately weighed sample was responded in dissolution media consisting 900 ml 0f 0.1 N (pH1.2) HCl and dissolution was done for 1hrs. The dissolution medium was then replaced with pH 4.5 phosphate buffer (900 ml) and drug release study was carried out for further 3 hr. After then the dissolution medium was replaced with phosphate buffer pH 7.2 (900 ml) and dissolution was continued for a further period of 4 hrs. Finally the dissolution medium was replaced with phosphate buffer 6.8 (900 ml) for a further period upto 24 hrs as the average residence time for intestine [14]. A sample volume of 1 ml was withdrawn from each dissolution vessel at regular intervals and replaced with equal volume of fresh dissolution medium. The sample was filtered and analyzed spectrophotometrically at 246 nm.

2.6 *In vivo* Anti- Inflammatory Study

Wistar albino rats of male sex were used for the study. Animals were maintained at temperature of 23 $\pm$ 1^0 C and 12 hr dark and 12 hr light cycle was followed, fed with standard pellet diet Animals were grouped in three groups, with six animals in each group. The groups comprised of normal or untreated animals, controlled group animals received 0.1 ml of 6% acetic acid. The third group received microspheres containing drug 2 mg/kg per day orally as suspension containing 0.5% CMC for 3 days. . After 48 hours animals were sacrificed by cervical dislocation, dissected, colon was flushed with normal saline solution. The colon was scored for inflammation based on microscopic features.

Sections were stained with haemotoxylin and eosin and microscopic assessment was done by light microscope. Histological damage was scored on 0-3 scoring pattern [15].

Kinetics of Drug Release: Data obtained from dissolution studies was fitted to various kinetic equations. The kinetic models were used zero order equation ($Q = Q_o - k_o t$), first order equation ($\ln Q = \ln Q_o - k_1 t$), Higuchi's equation ($Q = k_h t_{1/2}$) and Korsmeyer-Peppas equation, Log Q vs. Log t, where Q_t is cumulative amount of drug release at time t and Q_o is the initial amount of drug present in microspheres. k_o is the zero order release rate constant, k_1 is the first order release rate constant, and k_h is the diffusion rate constant. The coefficient of regression and release rate constant values for zero, first and Higuchi's and Korsmeyer- Peppas models were computed [16].

2.7 Stability Study

Stability study was carried on optimized formulation. The microspheres were wrapped in aluminium foil and placed in amber coloured bottle. It was stored at 40 ±2°C, 75% ±5% relative humidity for 3 months and evaluated for drug entrapment efficacy and in vitro drug release. The result obtained was compared with normal readings of same formulation.

3. RESULTS AND DISCUSSION

Tamarind gum microspheres were prepared by emulsion-dehydration technique.

Drug Excipient compatibility study: No considerable change in IR spectrum of pure drug and drug and excipient combination was observed so this study rules out any possibility of drug excipient interaction.

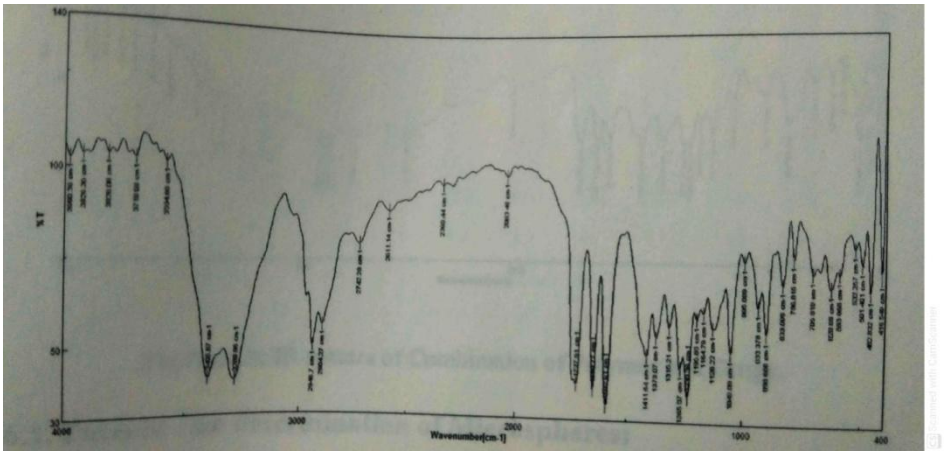


Fig. 1. FTIR spectra of Predinisolone

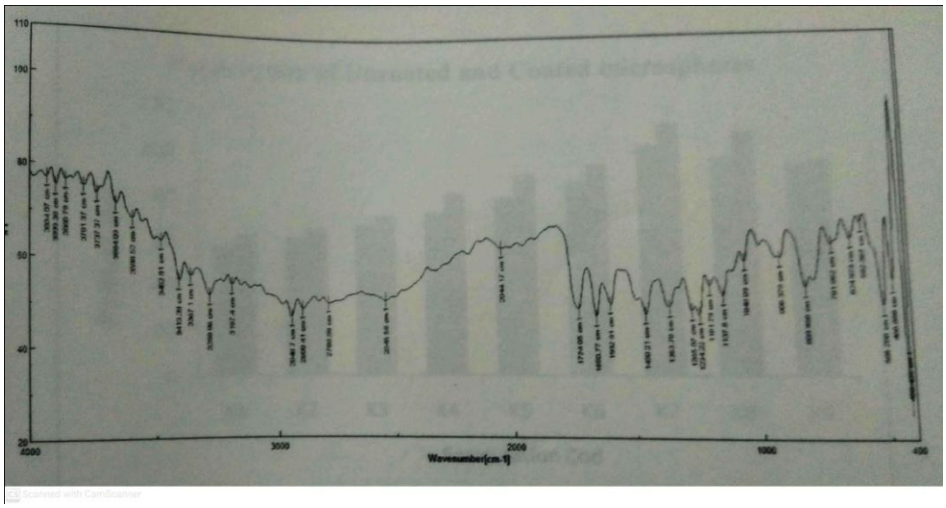


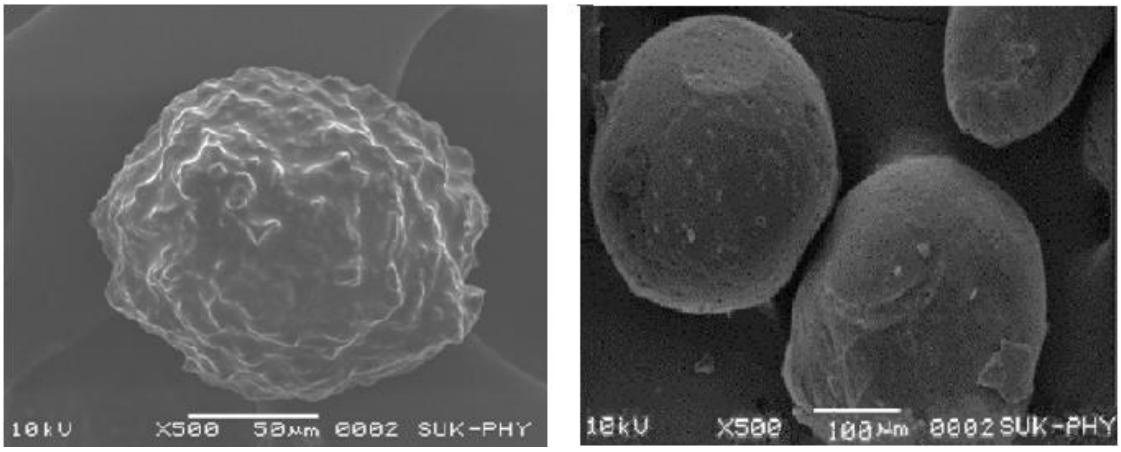
Fig. 2. FTIR spectra of drug and polymers

Surface Morphology of the Microspheres: The SEM analysis revealed that the shape and surface of uncoated microspheres prepared in this study were found to be spherical and rough in surface while Eudragit coated microspheres were found to be smooth in surface and spherical in shape.

Particle Size Distribution analysis: As the drug to polymer ratio was increased, the mean particle size of tamarind gum microspheres was also increased (Table 2). Mean particle size was found to be 57.32 µm with microspheres having 1:1 drug: polymer ratio while it was significantly increased to 102.59 µm up to the formulation T 7 having drug polymer ratio of 1:7. The significant increase may be because of the increase in viscosity of the droplets (due to increase in concentration of polymer solution). But further increase in polymer concentration results in difficult dispersion and thus subdivision of droplets may takes place, thus decreased in particle size (T8 and T9). The % yield increases with increase in polymer concentration. The increase in tamarind gum concentration increases entrapment efficiency.

The In vitro drug release of uncoated microspheres was carried out in different pH of gastrointestinal fluids. The effect of tamarind gum concentration was observed on in vitro drug release. Prednisolone release from tamarind gum microspheres in SGF was higher in formulation T1 with lower polymer concentration and decreases gradually till formulation T 8 The initial higher release of Prednisolone from microspheres might have resulted from the dissolution of drug on the surface of microspheres.

In case of batches with varying polymer concentrations, batch T1 released 17% in pH 1.2, the drug release lowered with increase in polymer concentration to 6% for T5.



(a) **(b)**

Fig. 3. SEM micrograph of microparticle prepared 1:5 drug: TKP ratio (a) uncoated microspheres (T5) (b) Eudragit S-100 coated microspheres (ET5)

Table 2. Mean particle size, % yield, and % drug entrapment efficiency

Formulation No.	Mean Particle Size (µm)	Percentage yield	Drug Entrapment Efficiency (%)	Formulation No.	Mean Particle Size (µm)
T 1	57.32 ±0.40	52.07	29.5 ± 1.20	ET 1	64.11 ±0.68
T 2	62.43 ± 0.38	53.57	32.65 ±1.28	ET 2	66.36 ± 0.76
T 3	68.44± 0.52	65.18	40.0 ± 1.36	ET 3	72.27 ± 0.76
T 4	73.63 ± 0.50	60.48	43.2 ± 1.26	ET 4	82.38 ±1.10
T 5	80.29 ± 0.54	57.52	48.71 ±1.28	ET 5	90.55 ±0.98
T 6	87.06 ± 0.52	57.83	52.3 ± 1.40	ET 6	94.52 ±1.20
T 7	102.59 ±0.6	55.48	58.5 ± 1.54	ET 7	112.49 ±1.10
T 8	96.22 ±0.56	54.57	62.2 ± 1.60	ET 8	108.06 ±1.22
T 9	92.82 ± 0.54	60.19	71.5 ± 1.58	ET 9	94.63 ± 1.10

In pH 4.5,at end of 4 hrs drug release was higher for formulation having lower polymer concentration, 38% , 32%, 22% , 19% and 16% for formulation T1,T2,T3, T4, T5 respectively.

The drug release in phosphate buffer pH 7.2 drug was 76% for T1, getting decreased to 46 for T5 while slight increase in release was seen for formulation T6 to T9.

The coating of Eudragit S-100 on tamarind gum microspheres was done using oil-in- oil solvent evaporation method. The results of in vitro drug release of multiparticulate system clearly revealed that Eudragit coated microspheres exhibited slow release, that is, only 14 – 20% drug releases in 4 hrs. While in simulated colonic pH buffer 7.2 drug release drug release was in range of 70% to 80% for the formulation T1 to T5 observed within 8 hrs, this could be due to dissolution of the Eudragit coat at pH 7.2 and on exposure of the tamarind gum microspheres were degraded and results higher percentage of drug release.

The formulations having higher percentage of polymer had shown the sustained type of release pattern. Thus, highest drug release from uncoated tamarind gum microspheres could be due to the fact that they are not able to maintain their integrity in upper part of GIT and show maximum release, while Eudragit coated tamarind gum microspheres maintain their integrity in upper part of GIT hence drug release was slow in comparison to uncoated microspheres. The decrease in drug release in initial phase proves the efficiency of pH sensitive coat for drug protecting drug release in upper GIT and higher release in the pH 7.2 and 6.8 pH proves the potential of formulation for colon specific drug release.

Thus Eudragit coated tamarind gum microspheres has the potential for targeting the drug to colon.

The result of animal study suggest that the animals treated with acetic acid for 4 days develop symptoms of acute colitis. Administration of drug loaded microspheres prevented the development of induced colitis, with suppression of diarrhea and rectal bleeding. Histological study suggest erosion, ulcerations and infiltrates of small round cells and sub-mucosal edema. Microspheres treated animals shown no sub-mucosal edema or abnormality of crypt cells.

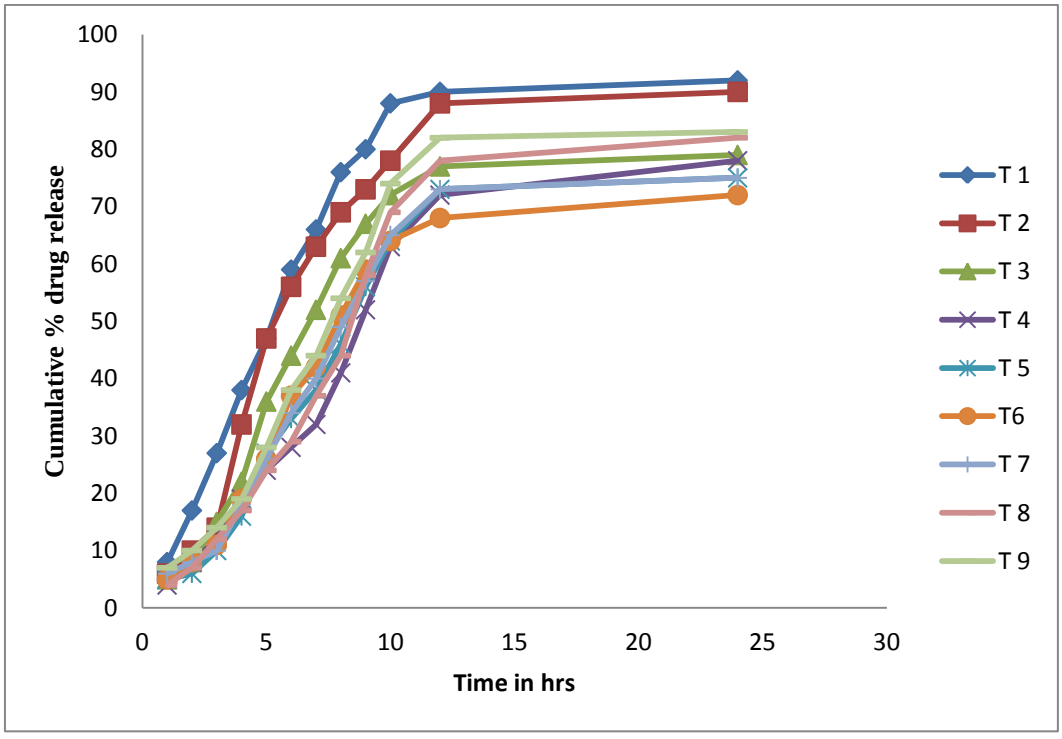


Fig. 4. Time Vs cumulative % drug release of uncoated microspheres

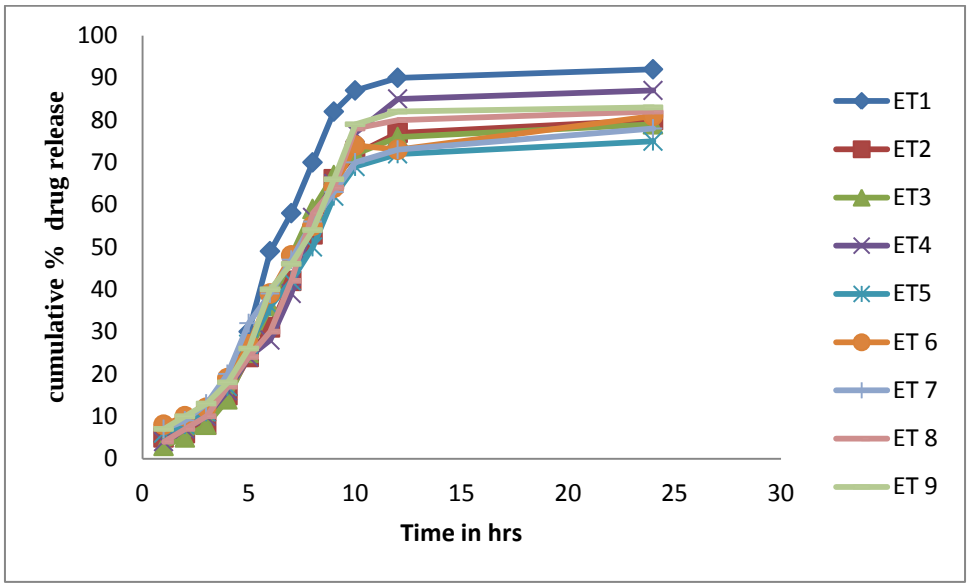


Fig. 5. Time Vs cumulative % drug release of eudragit coated microspheres

Histological Evaluation:

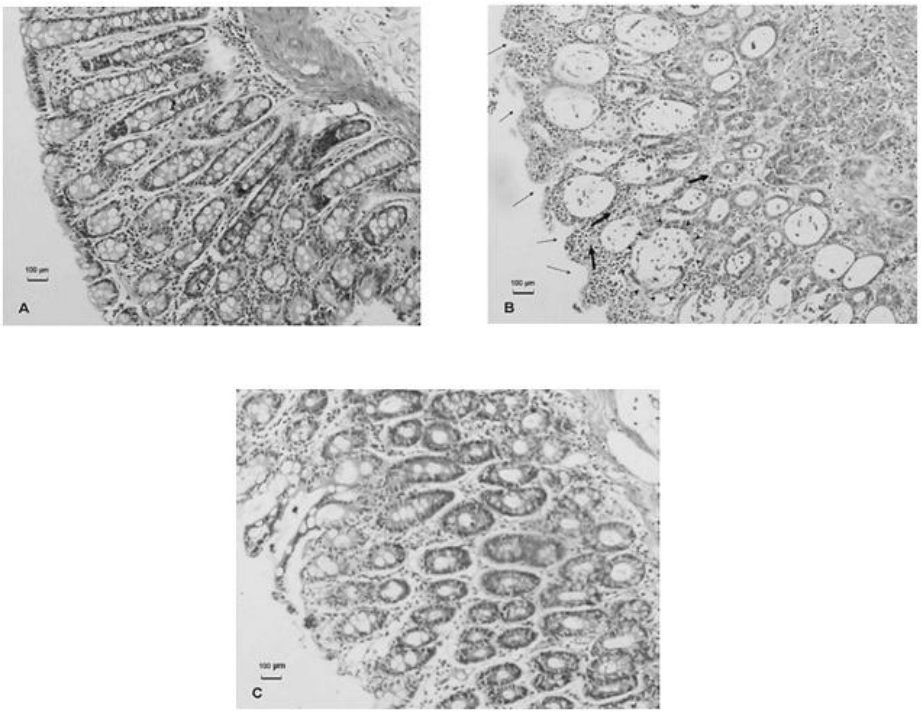


Fig. 6. (A) L.S. of control group rats (Acetic acid treated). (B) L.S. of drug containing microspheres treated rat. (C) L.S. of Prednisolone treated group rat

Stability Study

The stability study result indicates

1. No change in physical appearance of mictorspheres
2. The drug release pattern after stability study was nearly same as previous with slight difference.
3. The drug entrapment before stability was 48.71% ±1.28 and after stability was found to be 48.12% ±1.28

Kinetics of Drug Release: The results of kinetics data shows that in uncoated microspheres drug release follow zero order kinetics while the Eudragit coated microsphres followed Korsmeyer- Peppas equation. The n value determined for various formulations shows Non Fickian supper case II transport.

4. CONCLUSION

Formulation, Optimization and Evaluation of microspheres was carried out successfully The proposed emulsion dehydration technique is suitable for the microsphere preparation. Tamarind gum appears to have the potential for use in formulations. Results of invitro release study showed that the combination of enzyme biodegradability and pH sensitive coating presents promising approach for minimized release in gastric acidic environment and to extend higher release at higher pH region. The Eudragit S-100 coated tamarind gum microsphere containing Prednisolone, maintain its integrity in the stomach and small intestine and release of drug in colon. Hence Eudragit S-100 tamarind gum microsphere can be utilized and are having potential for the site specific delivery.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

In vivo study was done with study protocol as approved from IAEC having Approval no SCPM/IAEC/1112, Sahyadri College of Pharmacy, Methwade.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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