Original Research Article

Evaluation of *in vivo* efficacy and toxicity of prednisolone-loaded hydrogel-based drug delivery device

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Abstract

Drugs prescribed for the treatment of moderate to severe inflammatory bowel disease (IBD) are associated with number of side effects. Targeted drug delivery is essential for the treatment of inflammatory bowel disease in order to increase efficacy and reduce toxicity. The established delivery system is designed on enzyme and time-based release of poorly soluble prednisolone, a drug of choice for the treatment of moderate to severe inflammatory bowel disease. Their pharmacological evaluation was done in 2,4,6-trinitrobenzene sulphonic acid (TNBS)-induced model of colitis in rat. The drug was administered once daily for 3 consecutive days. Visible severity of colitis, tissue to bodyweight ratio, tissue histology along with nitric oxide (NO), malondialdehyde (MDA) and myeloperoxidase (MPO) activity of colonic tissue were studied to estimate the efficacy of the drug-loaded delivery system. The highest efficacy was observed for formulation in which Eudragit RS100 (EU) was used along with guar gum (GG) in a ratio 2:5 for the preparation of delivery device. An effective recovery was observed from the study of tissue histology of animals treated with the drug-loaded optimized formulation and the biochemical parameters supported it. The toxicity of prednisolone (PD) was reduced significantly as predicted from thymus to body weight ratio of treated animals. GG and EU RS100 provided a newer bipolymer combination for the colon-targeted delivery of PD which increased its efficacy and reduced the toxic side effects. The *in vivo* experiments presented effective amelioration from colitis in TNBS-induced animal model of colitis.

Key words: Colon, eudragit RS100, guar gum, prednisolone, TNBS colitis

INTRODUCTION

Inflammatory bowel disease including ulcerative colitis and Crohn's disease is becoming a severe, chronic, and refractory disease among the urban population in India.[1-4] The major causes may be attributed to various factors like genetic, environmental, and immune factors that affect the intestinal microflora and mucosal immune system.[5,6] Drugs of choice are mostly anti-inflammatory, antineoplastic drugs, and steroids that are readily absorbed from the upper part of the gastrointestinal tract (GIT) before reaching the desired site of action the colon and often get associated with multifarious side effects. There also several other drugs labile in the enzymatically active environment of the stomach and the small bowel. In order to formulate oral delivery system for these drugs, they need to be encapsulated within a delivery system that could protect the drug and allow its release in the colon. Site-specific delivery of an active pharmaceutical ingredient increases the bioavailability, reduces the amount of drug to be administered, and subsequently reduces the unwanted side effects. Prednisolone (PD) is the drug of choice prescribed in moderate to severe conditions of ulcerative colitis though it has number of side effects. PD is rapidly absorbed from stomach.[7] The biological $t^{1/2}$ for PD is 2.5 h,[8,9] but pharmacokinetics of the drug reportedly follows a nonlinear pattern[10,11] and the biological absorption is influenced by multiple factors including food intake throughout the GIT.[12] In systemic circulation, PD remains predominantly protein bound leading to series of side effects.[13,14] Thus, site-specific delivery of PD is expected to reduce these unwanted side effects.

Extensive literature survey revealed the use of both natural and synthetic polymers for the preparation of delivery systems. These include pH, time- and enzyme-dependent polymers, or various combinations of these polymers in the form of coating and matrix forming materials.[15-22]

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Our objective is to develop a colon-specific delivery system for PD using a combination of enzyme and time-dependent polymer for site-specific release of drug and effective amelioration of IBD. To formulate this drug delivery device we combined the properties of biodegradable polysaccharides with those of polyionic water insoluble pH-independent polymer with low porosity and inert to endogenous digestive secretions and enzymes.[23] Here, guar gum has been used as an enzyme-dependent polymer and Eudragit RS100 as the time-dependent polymer that is expected to provide a colon specific release of PD. An optimized formulation has been developed on the basis of *in vitro* drug release profile of the prepared formulations and other physicochemical properties.[24] This work is designed to study the *in vivo* efficacy and the usefulness of the site-specific drug delivery system for the treatment of inflammatory diseases of the colon.

MATERIALS AND METHODS

Materials

GG (mannose to galactose ratio 1:2) was purchased from Merck India Ltd., EU was purchased from Evonik India Ltd., PD was obtained as a gift sample from Dey's Medical Stores Mfg. Ltd., 2,4,6-trinitrobenzene sulphonic acid and reagents for biochemical analysis were purchased from Sigma Aldrich, USA. All other reagents and chemicals used were of Analytical Reagent grade (AR) and purchased locally.

Male Wister rats (6 weeks old, 200-210 g) were purchased from Central Ayurvedic Research Institute (Kolkata, India) and kept on normal diet with water *ad libitum*. The room temperature was maintained at 25 ± 1°C with relative humidity of 60% ± 5% and 12 h light-dark cycle. They were kept for 7 days without any treatment for acclimatization and from the 7th day onward they were used for the experiments. *In vivo* experiments were carried out in accordance with the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals under Ministry of Forests (Animal Welfare Division) for conducting animal experiments. All efforts were made to minimize animal suffering and to limit the number of animal used. The experimental protocol was approved by the animal ethics committee, Department of Chemical Technology, University College of Science and Technology, University of Calcutta, with registration number 506/01/aCPCSEA and proposal number 02 dated 09/02/2010.

Methods

The matrix tablets were prepared as reported previously.[24] Briefly, PD and different quantities of EU were dissolved together in 95% ethanol. Measured quantities of GG were mixed thoroughly with this solution. The mixture was dried for 30 min in a vacuum drier kept at 60 ± 1°C at 1 mm of mercury pressure. An aliquot of 5 % aqueous GG solution was then added to it as binder and the mass was kept for 4 h at room temperature (22°C). The dough was then passed through a No. 20 sieve and dried for 15 min at 105 ± 5°C. The granules, thus, obtained were compressed into tablets with fillers in a Minipress II MT tablet punching machine (Kalweka, Rimek, India).

As reported earlier,[24] the matrix tablets were characterized through infrared spectroscopy (FT-IR), powder x-ray diffraction study (PXRD), atomic force microscopy (AFM), and *in vitro* drug release studies. An optimized formulation was achieved through statistical evaluation of release data using factorial design.[24] The optimized formulation was further analyzed for its *in vivo* efficacy using rat model of colitis.

In vivo efficacy study

For *in vivo* efficacy study, a rat colitis model was developed using TNBS as the colitis inducing agent[25,26] with slight modifications. The treatment schedule was presented in Figure 1. The rats were fasted for 48 h with free access to water. TNBS was instilled into the rat's colon under light ether anesthesia. A baby feeding tube was inserted rectally into the colon in such a way that the tip was 8 cm proximal to the anus. Thereafter 0.6 mL of 5% w/v TNBS in 0.25 mL of 50% ethanol, resulting a total volume of 0.85 mL, was instilled into the lumen of the colon and the tube was flushed with 0.5 mL of air. This composition of TNBS solution gave a prompt inflammation in the prestudy experiment with uniform distribution throughout the colon and minimal mortality rate. Three days after TNBS treatment, the rats were weighed and those weighing 80-100% of their initial body weight (body weight before TNBS treatment) were used rejecting other rats from the *in vivo* studies.[26] Another two groups of animals (Groups I and II) were maintained under similar environmental conditions without TNBS treatment. Group I served as healthy group. In Group II, 0.85 mL of 50% ethanol was administered to each animal into the colon using the technique as mentioned earlier and served as the solvent control group.

The above-mentioned rats with TNBS colitis were randomly allocated into five groups with six animals in each group. The groups were a) Control group (Group III) received only 1.0 mL of saline, b) void delivery device (GG-EU) group (Group IV) received void delivery system (EU and GG hydrogel without any drug (30 mg/day), as a suspension in 1 mL saline), c) PD group (Group V) received 5 mg PD/(kg/day), d) prednisolone loaded in guar gum matrix (GG-PD) group (Group VI) 5 mg PD equivalent/(kg/day) in delivery system containing only GG and f) prednisolone loaded in guar gum-eudragit RS100 bipolymer matrix (GG-EU-PD) group (Group VII) received 5 mg PD equivalent/(kg/day) in optimized delivery system. Each of the formulations and PD was suspended in 1.0 mL of saline. These were administered using gavages once daily from the fourth day after TNBS colitis induction and continued for 3 consecutive days. Three days after drug treatment, that is, on the
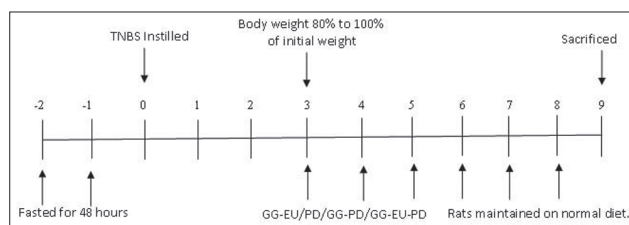

Figure 1: Schedule for the development of colitis model and subsequent treatment with healing drug

9th day, the rats were sacrificed using high dose of ether anesthesia. The large intestine, spleen, and thymus from each rat including the healthy and the solvent control group were excised and were immediately transferred into ice cold phosphate buffer saline and cleaned properly. The contents of large intestine were removed properly and cut open longitudinally in order to expose the mucosal surface. Extent of ulceration was noted. Each of these organs was weighed separately and kept refrigerated till further evaluation.

Severity of colitis was studied on the basis of visible damage to the colon, stool consistency, and rectal bleeding. The visual colonic damage score was carried out as reported earlier[27,28] on a 0-5 scoring basis. Briefly, a score 0 was given for no visible damage, 1 for localized hyperaemia or ulcer, 2 for linear ulcers with no inflammation, 3 for inflammation on site, 4 for two or more sites of inflammation or ulceration, and 5 for 1 cm long heavy ulceration along the length of the colon. For stool consistency, a score 0 was given for well-formed pellets, 2 for pasty and semiformed stool, and 4 for liquid stool that stuck to anus.[29] The scoring for rectal bleeding was 0 for no visible blood in stool, 2 for finding blood, and 4 for gross bleeding. A mean colonic damage (MCD) was calculated as a mean of all three scores.[26]

The body weight was taken for each animal before sacrifice. After sacrifice the organs, colon, spleen, and thymus were collected and the weight of each organ was recorded and their ratios to the body weights were determined.

Determination of biological markers in tissue homogenates

Another part of colonic tissue was washed thoroughly and homogenized in a tissue homogeniser in an ice-cold mixture of methanol and a 10% solution of trichloroacetic acid in the ratio of 1:1.5. The suspension was then centrifuged at 5000 rpm for 15 min in a cold centrifuge at 0°C. The supernatant was collected. Nitric oxide estimation was carried out using Griess reagent [1:1 mixture of sulfanilamide (1% w/v solution in 3M HCL) and 0.1% w/v solution of N-(1-napthyl) ethylenediammine dihydrochloride as mentioned elsewhere.[30-32]

Malondialdehyde was determined as an indicator for lipid peroxidation. MDA was quantified by the measurement of thiobarbituric acid (TBA) reactive species. The concentration of tissue MDA was estimated using a standard curve prepared by using tetraethoxypropane (TEP) and TBA.[33,34]

Estimation of myeloperoxidase activity can give us an idea about the extent of tissue inflammation. MPO was estimated from the colonic tissue homogenates in ice-cold phosphate buffer following O-dianisidine method as mentioned elsewhere.[35,36] For calculation one unit of MPO activity was considered to be equivalent to 1 μmol of peroxide consumed per minute at 25°C.

Plasma concentration of PD

Another set of rats were taken and as mentioned earlier after three days of TNBS treatment they were divided into two groups. They were fasted for 24 h. Then, PD alone was administered to the first group and GG-EU-PD to another group at a dose equivalent to 5 mg of PD per rat as a suspension in saline (1 mL per rat). Blood samples from the each rat were collected via jugular vein under light ether anesthesia immediately before and after 1, 2, 4, 8, 12, and 24 h. Plasma was collected after centrifuging the blood samples at 4000 rpm for 8 min in a cold centrifuge kept at 4°C. A total of 100 μL of saturated aqueous sodium chloride solution was mixed with 100 μL of plasma sample, 100 μL of 5% (w/v) phosphoric acid, and 4 mL mixture of tertiary butyl methyl ether and pentane (2:3, v/v) and the resulting mixture was shaken vigorously. Then, 3 mL of the organic phase was dried under nitrogen atmosphere at room temperature.[37] The resultant residue was dissolved in 1 mL of mobile phase and the amount of PD was analyzed by high performance liquid chromatography (HPLC).

Statistical analysis
All values were presented as mean ± standard deviation. Student's t-test and analysis of variance of the observed parameters were obtained to study the statistical significance of all data and a $P < 0.05$ was chosen as the level of significance.

RESULTS

Evaluation of prednisolone tablets
The drug contents of each of the nine formulations (T1-T9) were estimated using a validated HPLC method [Table 1]. An *in vitro* cumulative drug release study was carried out in simulated colonic fluid containing rat cecal contents [Figure 2]. Formulations T3, T6, and T9 showed minimal initial burst release and the optimum drug release was observed for formulation T9 throughout the drug release study. The FT-IR studies presented no significant drug-polymer interaction [Figure 3]. The powder XRD studies presented the entrapment of the drug in crystalline form within the polymer matrix [Figure 4]. The AFM studies presented the uniform distribution of PD within the polymer matrix when EU was used along with GG in the formulation of the matrix [Figure 5]. An optimized formulation was achieved through factorial design.[24] All *in vivo* experiments were carried out with the optimized formulation T9 [Table 1].

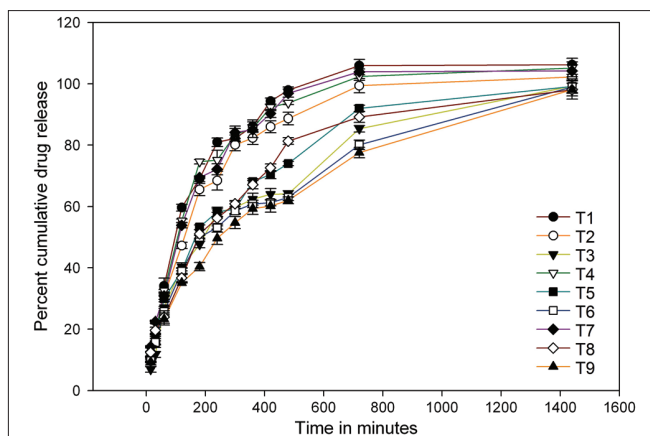| Table 1: Formulation, assay, and cumulative drug release of nine formulations				
Formulation	Amount of guar gum (mg)	Amount of Eudragit (mg)	Amount of drug (mg)	Assay % of total drug used in each formulation
T1	570	0	166	96.14
T2	570	160	166	98.12
T3	570	320	166	97.92
T4	720	0	166	96.74
T5	720	160	166	98.01
T6	720	320	166	98.32
T7	870	0	166	97.32
T8	870	160	166	96.99
T9	870	320	166	98.49

Figure 2: Cumulative drug release studies in simulated colonic fluid for formulations T1 to T9

In vivo efficacy study

Intracolonic administration of TNBS in 50% ethanol resulted in an inflammatory response characterized by extensive disruption of the colonic mucosa, linear deep ulcers, haemorrhage, and submucosal edema [Figure 6b]. Diarrhea and rectal bleeding were evident in all rats receiving no treatment [Table 2].

The MCD data represented the mean visual severity of colitis. The extent of formation of ulcer and the extent of healing on drug treatment can be understood from MCD data. The diarrhea and the rectal bleeding were reduced significantly in all three drug-treated groups, the maximum being for GG-EU-PD group [Table 2]. It was also found that equivalent amount of PD loaded in the delivery device have got a higher efficacy in the healing process than that of the drug alone or loaded in GG
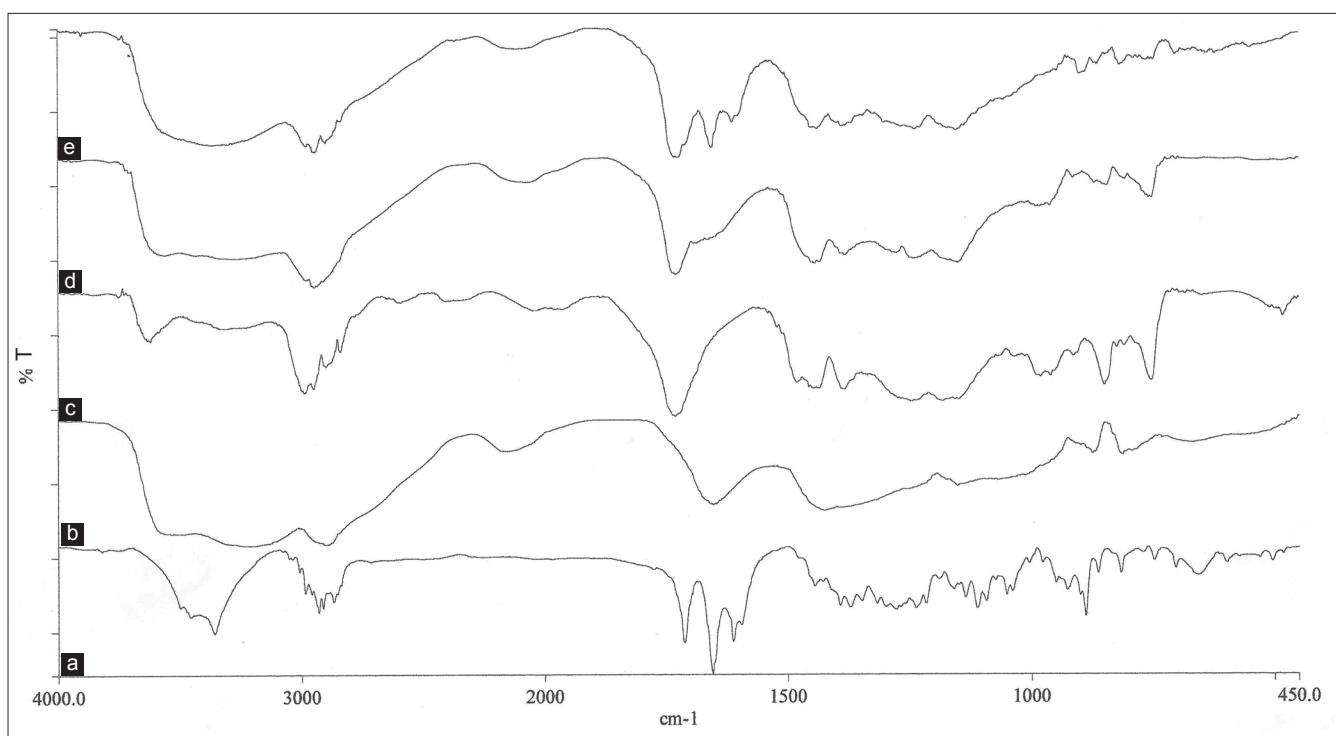

Figure 3: FTIR spectrum of prednisolone (a) guar gum (b) Eudragit RS100 (c) polymer combination (d) and formulation T9 (e)

Table 2: Effect of drug on visual severity in rats with 2,4,6-trinitrobenzene sulphonic acid-induced colitis

Treatment group	Visual severity of TNBS induced colitis			
	Visual colonic damage score [0-5]*	Stool consistency [0-4]*	Rectal bleeding [0-4]*	Mean colonic damage [0-3.77]*
Healthy	0	0	0	0
Solvent control	0	0	0	0
Control	4.92±0.20	4.00±0.00	1.10±0.90	3.36±2.90
GG-EU	4.50±0.45	4.00±1.80	1.00±5.90	3.33±4.86
PD	3.71±0.40**	2.00±0.30	0.70±0.40	2.42±3.60
GG-PD	3.75±0.42**	0.90±0.40§	0.30±0.70§	1.80±1.73‡
GG-EU-PD	1.83±0.52‡ §	0.30±0.50§	0.00±0.10§	0.82±0.76§

*Mean±standard deviation for six observations. §$P < 0.001$ as compared with the control group; **$P < 0.05$ as compared with the control group; ‡$P < 0.01$ as compared with prednisolone group. EU: Eudragit, GG: Guar gum, PD: Prednisolone
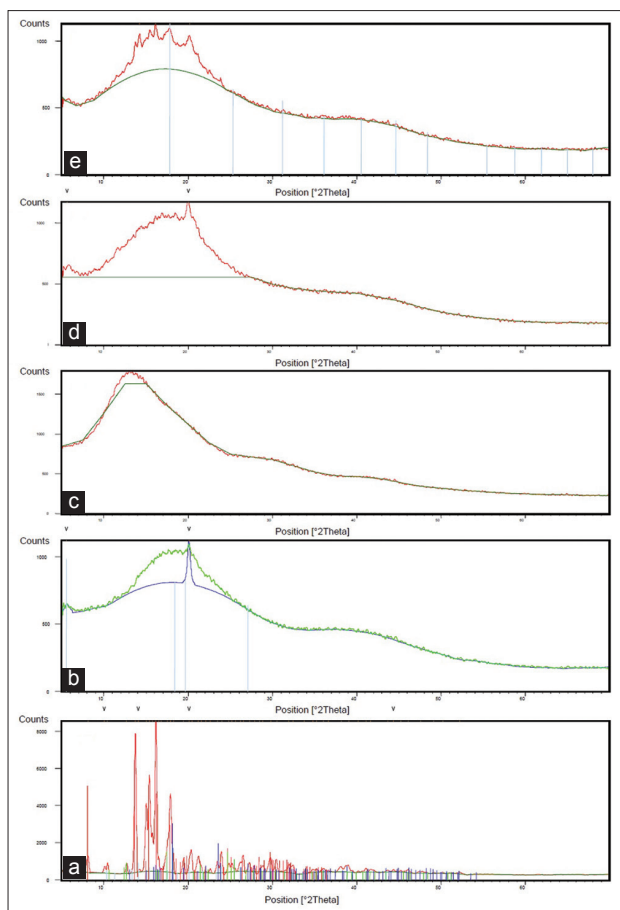
Figure 4: XRD spectrum of prednisolone (a), GG (b), Eudragit (c), polymer combination (d), formulation T9 (e)

matrix. The colon to body weight ratio also supported the above results [Table 3].

The value for GG-EU-PD group was close to the healthy group. The spleen to body weight ratio and thymus to body weight ratio also presented a similar ameliorative effect [Table 3].

There was no sign of ulcer formation in the colon of healthy animals. Formation of deep linear ulcer could be seen in the colon of the TNBS control group of animals. In case of PD group and GG-PD of animals, the extensive ulcer partially subsided. No sign of deep ulcer was observed in GG-EU-PD-treated group. Only mild inflammation of with signs of healing was observed visually [Figure 6].

Microscopic analysis of the colon tissue samples and histological assessment

Microscopic studies of haematoxylin and eosin-stained colon tissue sections of TNBS control group of animals showed extensive formation of ulcer with infiltration of inflammatory cells mostly neutrophils with moderate lymphoid tissue hyperplasia. However, no dysplasia of lining epithelium and fibrosis was observed. In GG-EU group ulcer formation with acute inflammatory cell infiltration was observed. In the PD group, acute inflammation partially subsided with partial healing of ulcer was observed. Partial healing was also observed in the colonic tissue section of GG-PD group. The GG-EU-PD-treated group showed moderate improvement [Figure 7]. The lymphoid tissue hyperplasia remained unaltered and mild development of dysplasia of the lining epithelium was observed in all the
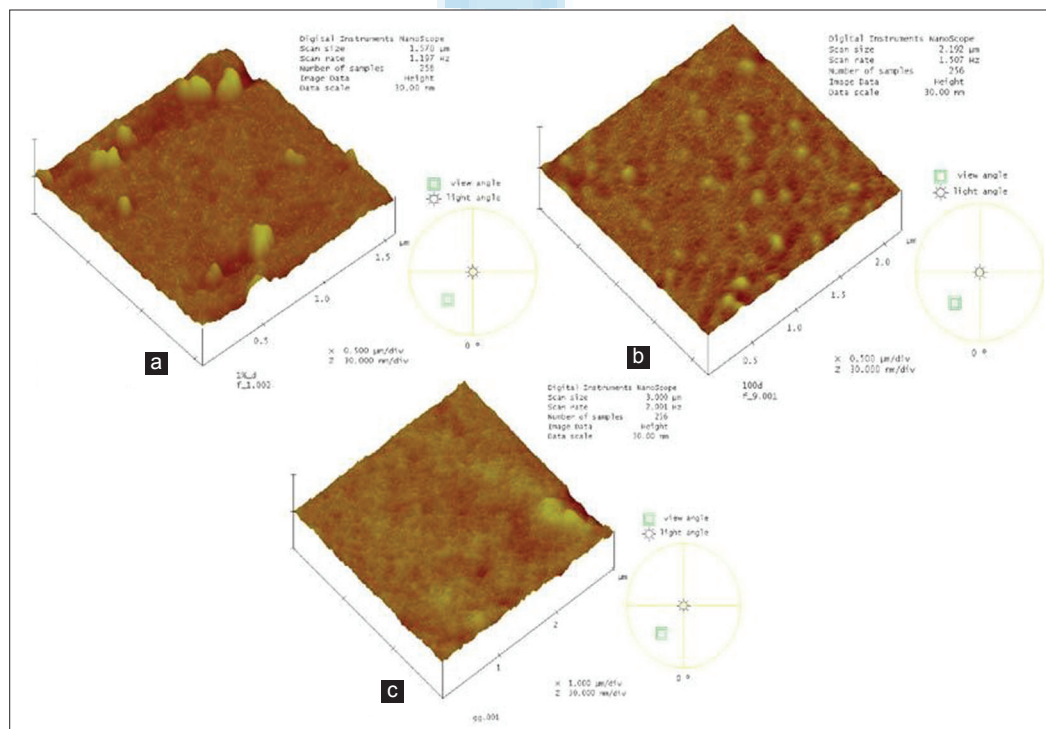

Figure 5: AFM images of formulation T1 (a), T9 (b), and Guar gum (c)

Table 3: Weight ratios of colon, spleen, and thymus to body weight

Treatment group	(Colon/body weight)*100	(Spleen/body weight)*100	(Thymus/body weight)*100
Healthy	2.17±1.67	1.80±0.89	0.30±0.12
Solvent control	3.01±1.98	2.09±2.01	0.27±0.09
Control group	6.96±4.89	2.57±1.67	0.09±0.05
GG-EU	7.01±3.99	3.56±1.89	0.10±0.07
PD	6.17±2.67*	2.38±0.59§	0.05±0.01*
GG-PD	5.99±2.78¶	2.12±0.67*	0.12±0.19*
GG-EU-PD	3.94±2.22¶	2.10±0.11*	0.29±0.09¶

The values are mean±standard deviation (*n* = 6 observations). *$P < 0.05$ as compared with the control group, ¶ $P < 0.001$ as compared with the control group, § the difference with control group is insignificant. EU: Eudragit, GG: Guar gum, PD: Prednisolone
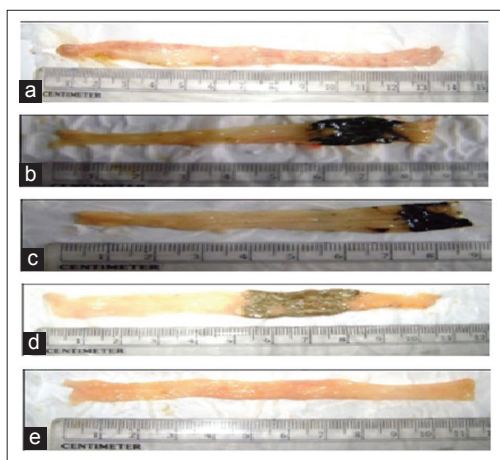

Figure 6: Representative longitudinal sectional view of colon of healthy rat (a), 2,4,6-trinitrobenzene sulphonic acid control (b), prednisolone (c), prednisolone loaded in guar gum matrix (d), and prednisolone loaded in guar gum-eudragit RS100 bipolymer matrix (e) group of rats
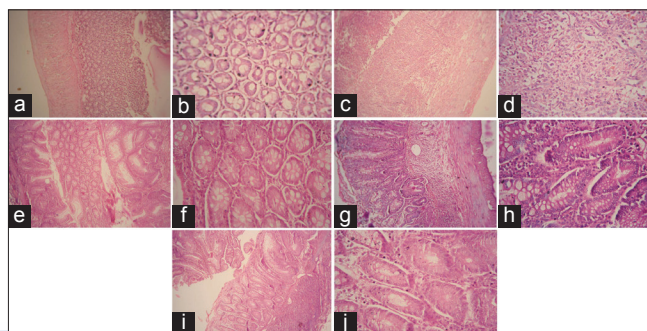

Figure 7: Representative histological appearance of rat colon: Normal colon of healthy rats at 10× magnification (a) and 40× magnification (b), Colon of 2,4,6-trinitrobenzene sulphonic acid-induced colitis group at 10× magnification (c), and 40× magnification (d), prednisolone treated colon of rats at 10× magnification (e), and at 40× magnification (f), prednisolone loaded in guar gum matrix-treated colon of rats at 10× magnification (g) and at 40× magnification (h), prednisolone loaded in guar gum-eudragit RS100 bipolymer matrix-treated colon of rats at 10× magnification (i), and at 40× magnification (j)

three drug-treated groups. The infiltration of inflammatory cells and severity of colitis was reduced significantly in case of the GG-EU-PD-treated group [Figure 7]. No fibrosis was observed in each group of animals. However, no ulcer formation was observed in the colonic tissue of healthy group and solvent control group, only mild reactive lymphoid tissue hyperplasia was observed.

Biochemical parameters

The studied biochemical parameters include tissue NO levels, MDA reactive species, and MPO activity. The results are tabulated in Table 4. Increase of NO generation has been reported in several models of experimental colitis[30] and IBD patients, and inhibition of nitric oxide synthetase (NOS) activity resulted in the amelioration of tissue injury in a model of rat colitis.[30-32] All these findings suggest that NO may have an effect on colonic inflammation, damage, and pathogenesis of IBD. The NO level in the tissue was significantly reduced on treatment with drug loaded in delivery system (1.91 n mole/g) compared to the control group with inflamed colonic tissue (3.49 n mole/g). However, the same was a still higher compared to the normal control (0.39 n mole/g).

MPO has been a plentiful constituent for neutrophils, its estimation can give us an idea of tissue neutrophil content.[35] The accumulation of neutrophil was a characteristic of inflamed tissue.[36] In the sites of inflammation, accumulation of neutrophil occurs to engulf or ingest the infective cells. The control group was also characterized by an increase of MPO activity which was reduced significantly for the GG-EU-PD group revealing lesser accumulation of MPO and initiation of healing. The value for MDA was also reduced significantly on drug treatment, the maximum reduction being for GG-EU-PD group 1.31 n mole/g [Table 3].

Plasma concentration of prednisolone

In order to study whether any portion of PD loaded in GG-EU-PD formulation enters into blood circulation, the dosage form was administered into TNBS instilled rats. PD alone at a dose of 5 mg PD equivalent/kg was administered to second group of animals to compare the effect of loading PD into the delivery system. The blood samples were collected and the plasma concentration of PD verses time graph was plotted as presented in Figure 4. It was observed that for free PD, the drug release took place almost instantly and it reached a value of 1.92 μg/mL

Table 4: Biochemical analysis of colonic tissue homogenates

Treatment group	NO (n mole/g of wet tissue)*	MDA (n mole/g of wet tissue)*	MPO Units/g of wet tissue
Healthy	0.39±2.89	0.43±2.41	0.15±0.32
Solvent control	0.48±2.01	0.55±1.77	0.19±0.89
Control group	3.49±2.38	7.60±1.2	0.46±0.38
GG-EU	3.93±2.98	7.89±0.89	0.44±0.24
PD	3.01±1.92[†]	4.56±2.01[§]	0.22±0.04[§]
GG-PD	2.89±1.54[§]	2.49±2.89[§]	0.23±0.19[§]
GG-EU-PD	1.91±2.57[§]	1.31±1.73[§]	0.16±0.04[§*††]

*The values are mean±standard deviation (n = 6 observations). [§]$P < 0.001$ in comparison with the control group, [†]$P < 0.05$ in comparison with the control group. *$P < 0.01$ in comparison with free prednisolone treatment, [††]$P < 0.05$ compared to prednisolone loaded in guar gum matrix group. EU: Eudragit, GG: Guar gum, PD: Prednisolone, MDA: Malondialdehyde, MPO: myeloperoxidase, NO: Nitric oxide
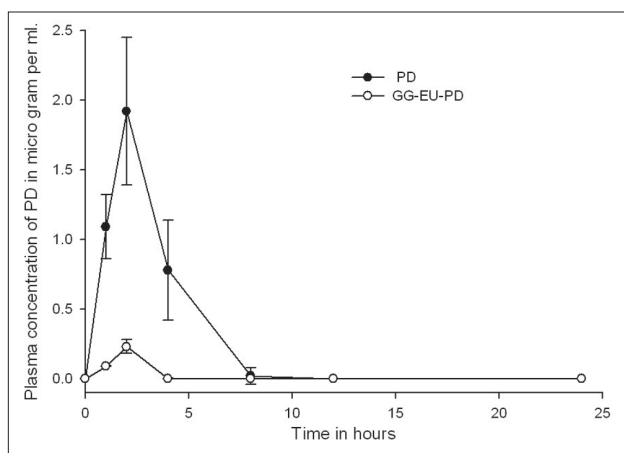

Figure 8: Plasma concentration-time graph of prednisolone after oral administration of prednisolone in delivery system and prednisolone alone. The data are represented as the mean ± standard deviation (n=3)

within 2 h that declined sharply and reduced to almost zero at the 8th h of study. After which, no further blood level of PD was observed up to 24 h. In case of GG-EU-PD, the plasma level of PD was very low of about 0.53 μg/mL after 2 h of study and it declined sharply to 0 μg/mL within 4 h of study [Figure 8].

DISCUSSION

The current study was conducted in order to develop a localized drug delivery system for PD. Anti-inflammatory steroids like PD was associated with a number of side effects and designing localized delivery system would reduce gastrointestinal absorption from undesired location of GIT.[17] In our study, the formulation batches were analyzed using a validated HPLC procedure and the drug load was found to be between 96.14% and 98.49% [Table 1]. The optimized formulation T9 achieved through factorial design[24] was used for in vivo efficacy study. The treatment of PD loaded in optimized drug delivery device on rat model of colitis revealed a possible localized delivery of the healing drug at the desired site of action. This markedly reduced the signs of inflammation and ulceration as revealed by macroscopic, histological, and biochemical parameters studied. The results were compared with PD alone or with PD loaded in GG. The macroscopic damage score was found to be much lower for GG-EU-PD group compared

with GG-PD group or PD group suggesting that greater amount of drug has been delivered to colon, resulting in better healing at the diseased site with GG-EU-PD group.[17] The colon to body weight ratio and thymus to body weight ratio for GG-EU-PD group was close to healthy group indicating the efficacy of PD loaded in GG and EU RS100 bipolymeric delivery device. Reduction in thymus to body weight ratio below those for normal animals was considered to be due to unwanted side effects of PD[36] which was observed with PD group. This was lower than the control group. Thymus to body weight ratio was higher for GG-EU-PD group than all other groups studied and was close to healthy group indicating the reduced toxic side effects of PD [Table 3]. Thus, it could be predicted that GG-EU-PD enhanced the efficacy of PD and reduced most of its toxic side effects.[17]

The histological study also supported the data with a clear sign of better healing of ulcer with GG-EU-PD group. The mild dysplasia of the lining epithelium may be due to rapid proliferation of the cells due to rapid healing of the damaged mucosa.

NO estimated from inflamed colonic tissue of TNBS control group of animals was much higher than normal. The same for GG-EU-PD group was close to normal group of animals. This indicates the reactive moieties of the active metabolites of nitrogen decreased sharply on GG-EU-PD[31] treatment. NO was also higher in the PD and GG-PD group since much of the drug may have been absorbed from the gut prior to reaching the colon with PD and GG-PD compared with the efficacious GG-EU-PD treatment.

It was also observed that TNBS-induced colitis results in drastic increase in tissue MDA concentration. With the administration of PD loaded in the GG-EU-PD delivery device, the same was lowered indicating a possible decrease in the disease severity.

MPO in the inflamed tissue was also reduced significantly for GG-EU-PD group indicating lesser presence of inflammatory cells and healing of the colonic mucosa.[35,36]

Thus, PD loaded in delivery system produced effective healing with reduced side effects.

The plasma concentration of PD in the GG-EU-PD-treated group was also negligible indicating the possible localized release

of the drug into the colonic mucosa before coming into blood stream. In case of PD group, the drug may have been released at the upper part of the GIT resulting in a sharp increase in the plasma concentration which also decreased sharply after getting protein bound,[7-9] thereby making it unavailable at the desired site of action.

CONCLUSION

The treatment with PD-loaded-optimized bipolymer combination markedly ameliorates the inflammation in rat model of colitis. The delivery system allowed the localized delivery and prevented the early release of PD. The tissue macroscopic and microscopic studies and the observed biochemical parameters predict the efficacy and potential of the colonic delivery system for PD along with a reduction of toxic side effects. This bipolymer combination can be further studied and used for the development of newer colon-targeted delivery systems for other drugs having therapeutic efficacy and toxic side effects.

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