Original Article

Proof-of-concept for Site-specific Delivery of Mesalamine Nanoparticles for Effective Therapy in Colitis

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ABSTRACT

Background: Mesalamine loaded polymeric nanoparticles were prepared using three different polymers namely, Eudragit RS100, PLGA (50:50), or Eudragit RLPO, with an aim of targeted delivery to the inflamed colon. **Materials and Methods:** Nanoparticles were prepared by modified emulsification solvent evaporation and characterized for various physicochemical characteristics viz., size, size distribution, mesalamine entrapment, and *in-vitro* release. **Results:** Amongst the various formulations prepared, formulation F5 mesalamine nanoparticles made with Eudragit RS100 showed drug entrapment of 72.09% with comparative discrete nearing spherical particle size of 200 nm. *In-vivo* targeting potential of nanoparticles to the inflamed tissue was evaluated in acetic acid-induced colitis rat model and efficacy was compared with pristine mesalamine powder. Biochemical estimations were carried in colonic tissue homogenate to check the oxidative damage. The myeloperoxidase activity and lipid peroxides were significantly decreased and glutathione content increased after the oral administration of mesalamine nanoparticles compared to pure drug mesalamine. **Conclusion:** This delivery system enabled the drug to accumulate in the inflamed tissue with higher efficiency than the pure drug thus nanoparticulate system was efficient in mitigating the experimental colitis.

Key words: Nanoparticles, Mesalamine, Inflammatory Bowel Disease, Eudragit RS100, PLGA, Eudragit RLPO.

INTRODUCTION

Inflammatory bowel diseases (IBD) involve several chronic inflammations of colon and pathologically classified as ulcerative colitis and Crohn's disease. In ulcerative colitis mucosa and submucosa of colon is inflamed whereas, in Crohn's disease entire gastrointestinal tract is inflamed, ileum being the primary site. In both these conditions, the common symptoms are diarrhoea, bloody stools, weight loss, abdominal pain, fever, and fatigue.[1,2]

Several drugs and therapeutic treatment modalities are available for IBD, however mesalamine; 5-aminosalicylic acid (5-ASA) remains the first-line treatment. Drugs used to treat patients with ulcerative colitis are not always effective because of nonspecific distribution, metabolism in the gastrointestinal tract, and side effects. One major therapeutic strategy consists of oral drug delivery with the selective release of entrapped active compounds to the colon.[3,4] Mesalamine exerts direct anti-inflammatory action on the colonic mucosa after getting converted to active drug through the action of colonic enzymes.

We need improved therapeutic system that will act in low dose and will have targeted delivery for improved therapeutic outcomes and patient

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compliance. Sustained release formulations like pellets, capsules, or tablets nay deliver the drug specifically in the colon for extended period. However, their efficiencies seem to be decreased in many cases due to diarrhoea, the most common symptom of BD. Diarrhoea causes early elimination of dosage form and reduces the contact time with the colonic mucosa.[4,5] The traditional treatment of IBD requires frequent intake of anti-inflammatory drugs at high doses. A carrier system that targets the delivery of drug in the inflamed regions for a prolonged period after oral administration would be ideal. Such therapeutic system would effectively reduce side effects and will also need lesser amount of drug.

Nanoparticulate systems offers advantages of modulation distribution and release profiles and also improves the therapeutic outcome efficiently.[6] Nanoparticles have shown potential for specific accumulation in areas with inflamed tissue due to enhanced permeability and retention effect which in turn reduce the side effects. Nanoparticles seems to be promising primarily to reduce the dosage frequency as system adheres to mucus layers due to their easier penetration. This accumulation of nano-sized drug delivery systems should locally deliver higher amounts of entrapped drugs to the inflamed areas, thus leading to better therapeutic efficacy and a decrease in systemic side effects.[6] An initial approach to target inflamed areas of the colon was to combine specific colonic drug delivery strategies with nanocarriers. Polymeric nanoparticles were chosen as carriers able to fulfil such criteria.

In the present study, an attempt is made to formulate and evaluate nanoparticles containing mesalamine for the treatment of inflammatory bowel disease. Designed a drug-containing nanoparticle with suitable polymer for their specific accumulation in the inflamed tissue in the colon to suppress inflammation and reduce the undesirable side effects of the drug. Here, formulated nanoparticles of mesalamine, characterized and explored their targeting potential for colon specific delivery.

MATERIALS AND METHODS

Materials

Mesalamine was a gift sample provided by Dr. Reddy's Laboratory, Hyderabad, India. Poly(D,L-lactide-co-glycolide) (PLGA) with a lactide:glycolide 50:50was purchased from Sigma-Aldrich. Eudragit RS 100 and Eudragit RLPO were the generous gift samples from Evonik Pvt. Ltd. Potassium bromide and Polyvinyl alcohol (PVA) was procured from Thomas Baker (chemicals) Pvt. Ltd. and all organic solvents were of analytical grade.

Preparation of nanoparticle using modified emulsification solvent evaporation technique

Mesalamine-loaded polymer nanoparticles were prepared using modified emulsification solvent evaporation method. Nanoparticles were prepared with three different polymers namely Eudragit RS100, PLGA 50-50, and Eudragit RLPO. Initially, nanoparticles were prepared using Eudragit RLPO with three different ratios namely 1:2, 1:4, and 1:6. Higher drug loading could be incorporated in 1:2. Hence 1:2 ratio was kept constant for the remaining two polymers. Dichloromethane was used as an organic phase and mesalamine and encapsulating polymer were dissolved in it. PVA was added as a surfactant in a fixed concentration of 3 % w/v in aqueous phase (Table 1). Both the phases were emulsified using high speed homogenizer at 18,000 rpm for 20 min followed by stirring at 12000 rpm for 20 min. The mixture was then stirred on magnetic stirrer for 3hr at 1000 rpm. The nanoparticles were separated by centrifugation for 30 min at 14,000 rpm. After centrifugation, the supernatant was used to know the un-entrapped drug. Pellet was reconstituted in water and washed free of drug and subjected to freeze-drying using 5% w/v of mannitol as a cryoprotectant.

Characterization of mesalamine nanoparticles

Fourier-transform infrared spectroscopy (FTIR) spectral studies

FTIR spectrum was recorded for pure mesalamine and the formulations. Briefly, the powdered sample was thoroughly mixed with potassium bromide and the mixture was then compressed into a transparent pellet under high pressure. The FTIR spectrum was recorded in FTIR spectrophotometer (Bruker, Germany).

Size and shape of nanoparticles

The size of the nanoparticles was measured using photon cross-correlation spectroscopy. Each sample

Table 1: Formulation composition of mesalamine nanoparticles for batch no. F1 to F5.

Ingredients	F1	F2	F3	F4	F5
Mesalamine	20	20	20	20	20
Eudragit RLPO	40	80	120	-	-
PLGA 50:50	-	-	-	40	-
Eudragit RS100	-	-	-	-	40
Dichloromethane	20	20	20	20	20
PVA Solution	3%w/v	3%w/v	3%w/v	3%w/v	3%w/v

was placed in transparent polystyrene cuvette (path length = 1cm) and placed in thermostatic sample chamber of size analyzer (Nanophox, Sympatec, Germany). Sample temperature was set at 25°C and 3 runs of 60 s were performed. Detection was carried out at a scattering angle of 90°C. From the resulting correlation curves, a 2nd order analysis was performed to calculate the mean particle size.[7,8]

The morphology of mesalamine loaded nanoparticles was analyzed using a scanning electron microscope (SEM). Samples for SEM were fixed on an aluminium stub using two-sided carbon tape and sputter-coated with a gold/palladium mixture (60:40) under vacuum in an argon atmosphere using a sputtering current of 40 mA. The samples were imaged using Scanning Electron Microscope (JEOL, JSM, Japan).[9]

Encapsulation efficiency (EE)

The amount of the drug entrapped within the nanoparticle was determined by measuring the non-entrapped (un-encapsulated) drug amount in the supernatant. The supernatant was collected after centrifugation and recovery of nanoparticles. The encapsulation was analyzed using validated UV-Vis spectrophotometric analysis at 330 nm. Encapsulation efficiency was determined by using the following expression.

$$EE\ (\%) = [(Wt\text{-}Wu)/Wt] \times 100$$

Where, Wt- the weight of the initial drug; Wu- the weight of the un-encapsulated drug.[10]

In-vitro mesalamine release studies

The *in-vitro* drug diffusion from the formulation was studied through an egg membrane using modified dissolution apparatus. Egg membrane, previously soaked overnight in the dissolution medium was tied to one end of a specially designed glass cylinder. 10 ml of the formulation was accurately placed into this assembly. The cylinder was attached to a stand and suspended in 100ml of dissolution medium maintained at 37 ± 5°C so that the membrane just touched the receptor medium surface. The dissolution was performed in 0.1 N HCl (pH 1.2) solution for 2 h followed by dissolution in phosphate buffer pH to 6.8, and then the release study was continued for an additional 3hr. After 3hr, the dissolution medium pH was adjusted to 7.4. The dissolution medium was stirred at low speed using a magnetic stirrer. Aliquots, each of 5 ml were withdrawn at hourly intervals and replaced by an equal volume of receptor medium. The aliquots were suitably diluted with receptor medium and analyzed by UV-Vis spectrophotometer at 330 nm. The quantity of drug equivalent to 20 mg of mesalamine nanoparticles was taken for diffusion study.[11]

Stability studies

Formulations were packed in an aluminum foil and sealed tightly and studies were carried out for 30 days by keeping at 4°C/Ambient, 40 ± 2°C and 75 ± 5% RH. The samples were withdrawn after 30 days and analyzed for the physical appearance and drug content.[12]

Therapeutic effect in colitis model

To study the effect of mesalamine nanoparticles on the inflamed tissue of the colon in ulcerative colitis, acetic acid-induced experimental colitis model was developed.

Animals and experimental design

All animal experiments were carried out in accordance with the recommendations for the use and care of laboratory animals by the Institute's Animal Ethics Committee (IAEC, approval no. DCD/GCP/20/E.C./ADM/12-13).

Sciences, US) Wistar rats (male, average weight 180-240 g, 10-12 weeks, *n*=24) were used. They were distributed in four different groups (*n*=6) as follows: Group 1; Normal or healthy control, Disease control or colitis induced, standard treatment group treated with pure drug Mesalamine, and test treatment group treated with mesalamine-loaded Eudragit RS100 nanoparticles). Animals were housed in an air-conditioned room at 22 ± 3°C, 55 ± 5% relative humidity, 12hr light/dark cycles, and allowed free access to water and laboratory chow for the duration of the studies.

Animals from group 3 and 4 were treated with mesalamine and mesalamine nanoparticles respectively for 7 days. On the 8th day, overnight fasted animals were anesthetized and A graduated rubber cannula was inserted rectally into the colon such that the tip was 8cm proximal to the anus. 2ml of 4%v/v acetic acid solution was instilled into the rectum. Acetic acid was instilled into the lumen of the colon through the rubber probe and 1 ml air was bubbled to spread the acetic acid solution in the colon. Animals were then maintained in a vertical position for 30 sec and returned to their cages. After 48hr of induction of acetic acid, animals will be sacrificed by cervical dislocation and dissected to remove the colon. Waste material will be removed from the colon by flushing with saline gently. The animals of standard and test group received orally plain solution of mesalamine and drug-loaded Eudragit RS100 nanoparticles (equivalent to 50mg/kg body weight) respectively. Oxidative stress markers, including Malondialdehyde (MDA) and anti-oxidant system markers, including superoxide dismutase

Table 2: Criteria for scoring the gross morphologic damage.

Score	Macroscopic changes
0	No visible change
1	Hyperaemia at sites
2	Lesions having a diameter of 1mm or less
3	Lesions having a diameter of 2mm or less (number < 5)
4	Lesions having a diameter of 2mm or less (number 5–10)
5	Lesions having a diameter of 2mm or less (number > 10)
6	Lesions having a diameter more than 2mm (number < 5)
7	Lesions having a diameter more than 2mm (number 5–10)
8	Lesions having a diameter more than 2mm (number > 10)

(SOD), myeloperoxidase (MPO), and glutathione (GSH) levels, were analysed. Histopathological examination was performed under light microscopy. Inflammation will be assessed based on physical parameters, and macroscopic features like colon weight and lesion score.

Macroscopic evaluation of colon for scoring of ulcers

The distal 10cm portion of the colon was removed and cut longitudinally, and slightly cleaned in physiological saline to remove fecal residues and pinned out onto a card. Macroscopic inflammation scores were assigned based on clinical features of the colon using an arbitrary scale ranging from 0–8 was used as follows (Table 2).[13,14]

Biochemical assays of tissue homogenate

Samples from the colon were immediately transferred to −80°C refrigerator and stored till analysis. Tissue samples were homogenized in 10 mM buffer (pH 7.4) and the homogenate was used for the measurement of MPO, MDA, SOD, and GSH.

Assessment of colonic MPO activity

Pieces of inflamed colonic tissue were rinsed with ice-cold saline, blotted dry, weighed, and excised. Minced tissue was homogenized in 10 volumes of ice-cold potassium phosphate buffer (pH 7.4), using a tissue homogenizer. The homogenate was centrifuged at 3500rpm for 30min at 4°C. The supernatant was discarded. 10ml of ice-cold 50mM potassium phosphate buffer (pH 6.0), containing 0.5% hexadecyl trimethyl ammonium bromide (HETAB) and 10mM EDTA was then added to the pellet. It was then subjected to one cycle of freezing and thawing and a brief period (15 sec) of sonication. After sonication, the solution was centrifuged at 15,000rpm for 20 min. MPO activity was measured spectrophotometrically as follows: 0.1 ml of supernatant was combined with 2.9ml of 50mM phosphate buffer containing 0.167mg/ml O-dianisidine hydrochloride and 0.0005% H_2O_2. The change in absorbance was measured spectrophotometrically, at 460nm.

One unit of MPO activity is defined as the change in absorbance per minute by 1.0 at room temperature, in the final reaction.[15,16]

Calculation of MPO activity

$$\text{MPO activity (U/g)} = \frac{x}{\text{Wt of the price of tissue taken}}$$

where

$$x = \frac{10 \times \text{change in absorbance per minute}}{\text{Volume of supernatant taken in the final reaction}}$$

Measurement of colonic MDA

Lipid peroxidation, an indicator of mucosal injury induced by reactive oxygen species was measured as a thiobarbituric acid reactive substance. The extent of lipid peroxidation is measured by estimating the amount of MDA. The amount of colonic lipid peroxides was measured by the thiobarbituric acid (TBA) assay. Briefly, 0.1ml of colonic tissue homogenates prepared were reacted with 1 ml of TBA reagent containing 0.375% w/v TBA, 15%w/v trichloroacetic acid and 0.25 N HCl (1:1:1). Samples were boiled for 30min, cooled by placing in crushed ice for 10 min and centrifuged at 6000rpm for 10min. Absorbance of the supernatants clear pink color was spectrophotometrically measured at 532nm against appropriate blank.[15]

Determination of colonic GSH contents

This assay is based on the formation of a relatively stable yellow product when sulphydryl groups react with 5, 5-dithio- 2-nitrobenzoic acid (DTNB). Briefly, 0.5ml of the homogenate is mixed with 0.1ml of 25 % w/v TCA to precipitate proteins and centrifuged at 4000rpm for 5 min. Then 0.3ml of the supernatant was mixed with 0.5 ml of 0.1 M phosphate buffer (pH 7.4) and 0.2 ml of 10mM DTNB. This mixture was incubated for 10 min and the absorbance was measured at 412 nm against appropriate blanks.

Histological Analysis

After sacrifice, colons were removed and washed with a mixture of PBS (pH 7.4) containing EDTA (1 M), harvested, and fixed in 4% formalin in normal saline,

approximately 10hr at 4°C. Later, dehydration was performed using absolute ethanol and coated with liquid paraffin. It was also cut into sections (3 µm) in a microtome and stained using hematoxylin and eosin for observing inflammatory cell infiltration and colonic mucosal damage. Under a light microscope using OLYMPUS Stream image analysis, inflammatory cell infiltration, mucus production, and hemorrhagic damage were observed.

Statistical analysis

Results were expressed as means ± S.E.M. The statistical significance of any difference in each parameter among the groups was evaluated by one-way ANOVA, using Tukey–Kramer multiple comparisons test as a *post hoc* test. *P* values of < 0.05 were considered statistically significant.[17]

RESULTS

Fourier-transform infrared spectroscopy (FTIR) spectral studies

Mesalamine-loaded polymer nanoparticles were prepared by modified emulsification solvent evaporation technique. Nanoparticles were prepared with three different polymers namely Eudragit RS100, PLGA 50-50, and Eudragit RLPO. Initially, drug to Eudragit RLPO were tried with three different ratios i.e., 1:2, 1:4, and 1:6. More drug could be incorporated in a 1:2 ratio. Hence 1:2 ratio was kept same for the remaining two polymers as well.

Drug polymer compatibility studies were carried out using FTIR to establish the possible interaction which would be reflected by a change in the position or disappearance of any characteristic peaks of the compound. FTIR spectrum was recorded for mesalamine, drug loaded Eudragit RLPO, Eudragit RS100 and PLGA nanoparticles. The main peaks obtained from the combinations were similar to that of pure drug mesalamine and showed no shift and no disappearance of characteristic peaks suggesting that there is no interaction between drug and polymer as depicted in Figure 1.

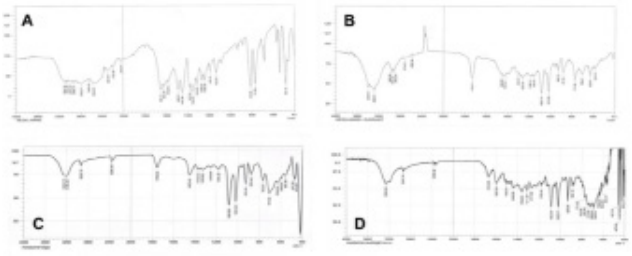

Figure 1: FTIR spectra of A. Pure drug mesalamine, B. Mixture of mesalamine and Eudragit RLPO, C. Mixture of mesalamine and PLGA, and D. Mixture of mesalamine and Eudragit RS100.

Table 3: Particle size (nm) and mesalamine entrapment efficiency (%).

Formulation	Drug entrapment efficiency (%)	Mean Particle size(nm)
F1	68.03	174.12
F2	79.76	186.9
F3	82.3	135.4
F4	65.45	397.6
F5	72.09	240.2

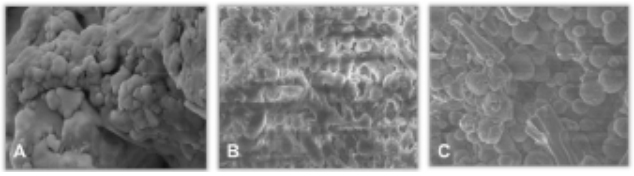

Figure 2: Scanning electron microphotographs of formulation A. F1, B. F4 and C. F5.

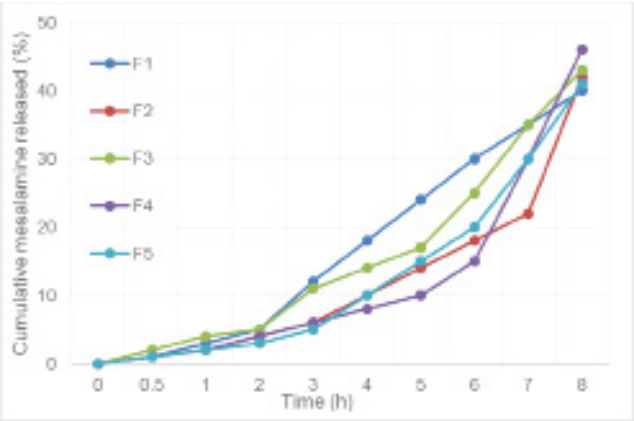

Figure 3: *In-vitro* drug release profile of all the formulation F to F5 (Mean of six determinations).

Mesalamine entrapment efficiency, size and shape of nanoparticles

Table 3 shows that the % drug entrapment efficiency and mean particle size (nm) of all the formulations was found to be varied from 65.45% to 82.3% and 135.4nm to 396 nm respectively

The morphology of the nanoparticles was investigated by scanning electron microscope (SEM). The photographs of the optimized formulations taken by scanning electron microscope are depicted in Figure 2.

The results of SEM revealed that nanoparticles of F2 and F4 formulations showed agglomerates with irregular particle shapes. Formulation F5 of nanoparticles showed comparatively discrete nearing spherical shape.

Mesalamine release from nanoparticles

The *in-vitro* release profiles of mesalamine from the prepared particulate system were studied in gradually pH changing buffers. The release of mesalamine from the nanoparticles was characterized by an initial phase of burst effect at pH 7.4, followed by the second phase of moderate release as depicted in Figure 3.

Stability studies of mesalamine nanoparticles

Stability studies of the nanoparticles were carried out, by storing formulations at 4°C in the refrigerator, ambient temperature, and humidity, and 40 ± 2°C / 75 ± 5% RH in humidity control oven for thirty days. Two parameters namely drug content and color of the preparation were monitored**.** Formulations did not show any change in appearance at any of the storage conditions in regards to the clarity and color of the preparation. There was no change in drug content in the formulation stored at 4°C. The formulation F5 had 40 to 200 nm particle size with entrapment efficiency of 72.09% was considered as the better nanoparticle formulation for animal study.

In vivo efficacy of mesalamine nanoparticles in ulcerative colitis model

To study the potential of developed formulation for targeted oral drug delivery to the inflamed tissue of the colon in UC, acetic acid-induced experimental colitis model was selected. Figure 4 are the photographs of the colon isolated after the competition of study. There was an increase in the macroscopic score, indicator of tissue damage (ileum) of rats treated with the control group as compared to the normal group.

Test and the standard group were effective in acetic acid-induced ulcerative colitis in rats. Evaluation based on macroscopic features showed lower score values for the test group and standard group when compared to the control group alone treated group. Score values of the treated test group were comparable with scores obtained in the standard group as depicted in Figure 5A.

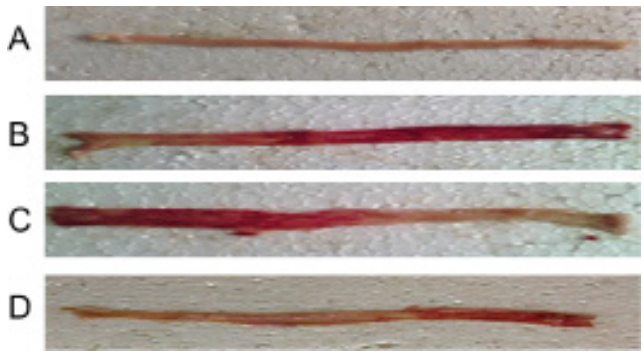

Figure 4: Representative pictures of colon A. Normal group, B. Disease control, C. Diseased group treated with mesalamine drug, and D. Diseased group treated with mesalamine eudragit RS100 nanoparticles (F5).

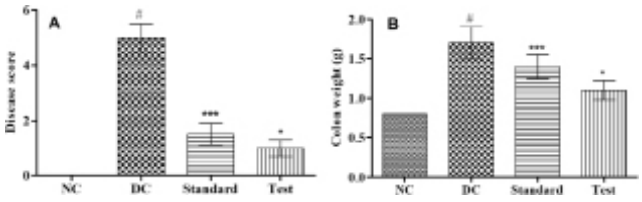

Figure 5A: Macroscopic scoring of colon for disease severity index and B. Colon weight. All values are represented as mean ± SEM, *n*= 6, #*p*<0.001, ***p*<0.05, **p*<0.05, when all groups were compared with normal control group.

Biochemical analysis of colonic tissue homogenate

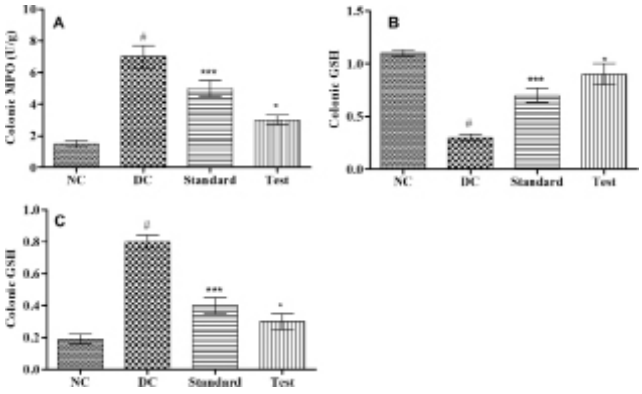

Figure 6A: Demonstrates the increased mucosal MPO concentration in the colonic mucosa of rats following intrarectal administration of the control group.
Figure 6: Biochemical estimations in colonic tissue extracts A. MPO activity, B. GSH content, and C. MPO. All values are represented as mean ± SEM, *n*= 6, #*p*<0.001, ***p*<0.05, **p*<0.05, when all groups were compared with normal control group.

Figure 5B shows control group produced a significant increase in colonic weight compared with the normal group. Test group treatment significantly showed decreased colonic weight as compared with the control group. The standard group also protected against induced by acetic acid but to a lower degree than the test group.

DISCUSSION

Preliminary experiments were carried out to ensure the compatibility of selected polymers with mesalamine using FTIR studies. The spectra revealed the compatibility of polymers with drug. Peaks of mesalamine (Figure 1A) at 3486, 3002, 1611, 1445, 1348, and 1130 cm^{-1} are observed in FTIR spectra of physical mixtures of mesalamine with encapsulating polymers. So, the results

confirm that mesalamine is not structurally modified and is compatible with the selected polymers.

We employed emulsion solvent evaporation technique for the preparation of nanoparticles as the technique is successfully applied for biocompatible polymers such as PLGA and Eudragit.[18] The technique offers several advantages over other methods such as spray drying, and homogenisation. Emulsion solvent diffusion requires mild processing conditions and low energy mixing. Mesalamine nanoparticles prepared with Eudragit (batch no F5) showed more than high drug entrapment and particles were also discrete (Figure 2C).

As the entrapment efficiency was increased, the extent of drug release decreased. A significant decrease in the rate and extent of drug release is attributed to the increase in density of the polymer matrix. Additionally, the larger particle size at higher polymer concentration also restricted the total surface area resulting in the slower release. The formulations F1, F2, F3, F4, and F5 showed cumulative drug release of 43.98% to 51.91% at the end of 8hr. The *in-vitro* dissolution studies of mesalamine from the prepared nanoparticles of formulations F1, F2, F3, and F4 exhibited a sustained release. The *in-vitro* dissolution studies of mesalamine from the F5 nanoparticles exhibited a biphasic pattern. Therefore, we explored this batch for *in vivo* efficacy in acetic acid induced colitis model.

Macroscopic indexing helped us to determine the extent of damage in intestinal mucosa in colitis model study. Figure 4 shows gross morphological changes on the colon of various groups at the end of study period. The normal control group had no pathological changes, whereas, the diseased control group showed significant increase in macroscopic score marked by oedema, necrosis, ulceration and hyperaemia as shown in Figure 5A and B. Diseased animals treated with mesalamine nanoparticles showed improvement and it was significantly faster than the pure drug treatment.

In regard to the biochemical changes, pre-treatment with either standard or test group produced a significant reduction in MPO activity as compared to the control group but was still higher than the normal group. Figure 6B shows control group produced a significant decrease in colonic GSH content compared with the normal group. Test group treatment significantly increased GSH content as compared with the control group. The standard group also protected against GSH depletion induced by acetic acid but to a lower degree than the test group. Figure 6C shows that colonic lipid peroxides concentration in the control group increased in comparison to the normal group. Treatment of rats with mesalamine nanoparticles (test group) produced a marked significant decrease in lipid peroxides concentration that reaches the normal group. The standard group also provided protection against the elevation in lipid peroxides concentration induced by the control group.

IBD is a complex, multifactorial, gastrointestinal disease with chronic relapsing inflammation in the intestine. It is the most challenging human disease to treat because of several limitations of conventional drug delivery system. Nanotechnology offers very promising outcomes,[19] in the treatment of IBD by virtue of their small particle size and ability to get targeted to the inflamed tissue because of enhanced permeability and retention effect. In this context, our current investigation provides a proof-of-concept for the preparation of mesalamine nanoparticles and their therapeutic efficacy in colitis.

CONCLUSION

In the present work nanoparticles containing mesalamine were formulated to treat inflammatory bowel disease. The preparation and evaluation parameters of the formulations have been discussed in the previous chapters. From the study following conclusions could be drawn. The results of this investigation indicate that nanotechnology can be successfully employed to fabricate mesalamine nanoparticles using three different polymers with a modified emulsification solvent evaporation technique. FT-IR spectra of drug and formulation revealed that the drug is compatible with the polymers used for the formulations. SEM analysis of the nanoparticles revealed that drug-loaded eudragit RLPO and PLGA nanoparticles showed 200-400nm particle size with agglomerates with irregular surface whereas drug-loaded eudragit RS100 nanoparticles showed comparatively discrete nearing spherical shaped particles size ranging from 40-200nm without agglomerates which were further used for *in-vivo* studies. The drug diffusion of eudragit RLPO and PLGA showed sustained release of drug whereas eudragit RS100 was characterized by an initial phase of burst release followed by a second phase of controlled release. Administration of eudragit RS100 nanoparticles (formulation F5) to animals with experimentally acetic acid-induced colitis led to a significant recovery of induced inflammation. Application of free mesalamine was also shown to be effective, but to a lesser extent than mesalamine delivered in eudragit RS100 nanoparticles. From a future perspective, further investigations for clinically viable Nano-carriers are essential. Also, based on these investigations, further clinical studies on human patients are required

to achieve the translation of nanoparticle-based therapeutics and their associated advantages from the laboratory into the clinic.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

IBD: Inflammatory Bowel Diseases; **5-ASA:** 5-aminosalicylic acid; **PLGA:** Poly(D,L-lactide-co-glycolide); **PVA:** Polyvinyl Alcohol; **FTIR:** Fourier-Transform Infrared Spectroscopy; **SEM:** Scanning Electron Microscope; **EE:** Encapsulation efficiency; **MDA:** Malondialdehyde; **SOD:** Superoxide dismutase; **MPO:** myeloperoxidase; **GSH:** Glutathione; **HETAB:** Hexadecyl trimethyl ammonium bromide; **TBA:** Thiobarbituric acid; **DTNB:** 5, 5-dithio- 2-nitrobenzoic acid.

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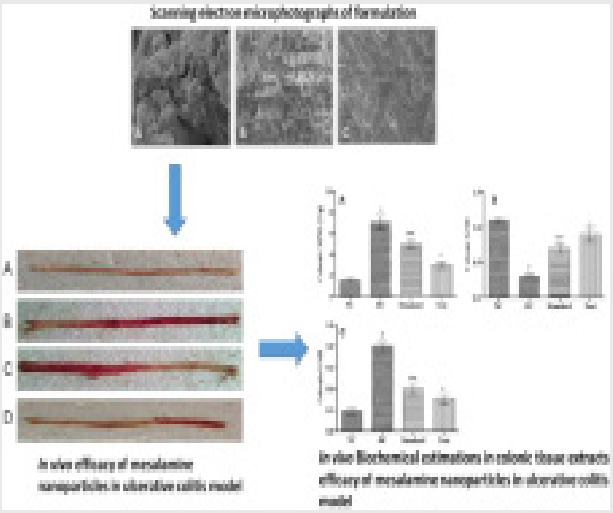
PICTORIAL ABSTRACT



SUMMARY

Nanotechnology offers very promising outcomes, in the treatment of IBD by virtue of their small particle size and ability to get targeted to the inflamed tissue because of enhanced permeability and retention effect. In this context, our current investigation provides a proof-of-concept for the preparation of mesalamine nanoparticles and their therapeutic efficacy in colitis. Also, based on these investigations, further clinical studies on human patients are required to achieve the translation of nanoparticle-based therapeutics and their associated advantages from the laboratory into the clinic.

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