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Anti-oxidant and anti-inflammatory potential of different polymer-based mesalamine delayed-release granules in TNBS-induced ulcerative colitis in wistar rats

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ABSTRACT

Ulcerative colitis (UC) is an inflammatory condition of colon characterized by severe damage to the innermost colon tissues. A number of studies described the use of medication delivery systems based on natural polymers like polysaccharides for the purpose of reaching the colon. In this research, polymer-based mesalamine delayed-release granules (DRGs) were tested for their antioxidant and anti-inflammatory efficacy against UC. Chitosan (C), pectin (P), and pectin-chitosan (PC) mesalamine (M) DRGs were prepared and characterized. Data revealed satisfactory compatibility, flow, packing properties, drug release pattern, and delayed drug release by DRGs. Wistar rats were treated with 2,4,6-trinitrobenzenesulfonic acid (TNBS) (100 mg/kg) *via* rectal administration. Mesalamine and mesalamine DRGs (50 mg/kg) were administered orally separately for 14 days. Biomarkers of oxidative stress, inflammation, hematological tests, colon profile, and histopathology were performed. The findings demonstrated the good efficacy of the polysaccharides in delivering mesalamine to colon. Mesalamine and mesalamine DRGs based on various polymers showed significant antioxidant and anti-inflammatory effects in rats with UC. Mesalamine granules significantly attenuated colon lipid peroxidation, nitrites, myeloperoxidase activity, and interleukin-1β levels, and improved anti-oxidants (GSH, SOD). Data showed upregulation of Nrf2 activity by mesalamine granules with CM-DRGs showing maximum effect. Mesalamine and different polymer-based mesalamine DRGs significantly attenuated TNBS-induced decline in body weight, ulcer severity, and colon damage. CM-DRGs showed the most pronounced ameliorative effect on colon and hematology parameters via anti-oxidant and anti-inflammatory activities. Chitosan can be used as a carrier for oral colon delivery of mesalamine in DRG formulation for enhanced therapeutic efficacy in UC.

1. Introduction

Ulcerative colitis (UC) is an idiopathic illness affecting the area between the proximal colon and rectum with different severity. The etiology of UC is undefined and multifactorial including several environmental factors, host-immune dysfunctions, and intestinal dysbiosis in genetically vulnerable persons (Gajendran et al., 2019). It is a progressive form of inflammatory bowel disease (IBD) with relapsing and remitting course patterns. The risk of UC is greatest in age groups between 20 and 30 and 50–80 years of age. Weight loss, colonic mucosal

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ulcers, abdominal pain, and bloody or mucous-rich diarrhea are the classic clinical symptoms of UC. Biopsy, endoscopy, and histology are the primary diagnostic tools to assess the severity and range of colon damage in UC (Du and Ha, 2020). Inflammation in the deeper layers of the colon, epithelial and mucosal damage, and ulceration are driven by a hyperactivated immune response and aberrant CD4 + T-helper cell-mediated immunity. Th1 and Th2 maintain the inflammatory conditions in the colon. However, recent studies established that several other pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukins (ILs) (*e.g.*, IL-1, IL-1β, IL-6, IL-12), and interferon (IFN)-γ along with oxidative stress are responsible for induction and remission of UC (Gomez-Bris et al., 2023).

Treatment of UC focuses on the lower part of the large bowel which can be targeted by localized drug delivery in the colon (Kayal and Shah, 2019). Several physiological hurdles, including different pH, transit time, microflora, drug breakdown, and absorption in the stomach and small intestine, must be overcome for the drug to reach the colon (Ibrahim, 2023). Often drug delivery in the colon is hampered by several gastrointestinal (GIT) disorders. Therefore, various approaches have been explored for drug delivery in the colon, with each relying on one or more of the following fundamental mechanisms: prodrugs, time-dependent formulations, pH-triggered polymeric systems, and microflora-activated drug release by biodegradable polymers (Teruel et al., 2020). In the present research, we targeted a microflora-activated system for oral colon-targeted drug delivery. The proximal section of the large intestine is a common habitat for a number of anaerobic bacteria. For their sustenance, these bacteria rely mostly on carbohydrates. Polysaccharide-based medication delivery in the colon relies on the presence and activity of colonic bacteria such as *Bacteroides*, *Bifidobacterium*, *Eubacterium*, and *Lactobacillus* (Zhang et al., 2021). The drug is released only when polysaccharide carriers are digested by the colon bacteria in the colon.

Mesalamine (5-aminosalicylic acid) is the 1st line therapy of IBD, often accompanied by various adjunct therapies such as corticosteroids and anti-inflammatory drugs (Aslam et al., 2022). The exact mechanism of mesalamine (M) is unknown so far, however, it is acknowledged widely that mesalamine targets *cyclo*-oxygenase and lipo-oxygenase pathways to reduce the biosynthesis of prostaglandins and leukotrienes which are chief mediators of inflammation. Additionally, peroxisome proliferator-activated receptor (PPAR)-γ is also implicated in the anti-inflammatory activity of mesalamine (Chibbar and Moss, 2020). Due to the direct impact of mesalamine on the inflamed mucosa, a greater concentration of mesalamine inside the mucosa is necessary to provide a more significant therapeutic effect (Ferretti et al., 2022). Since mesalamine is absorbed in upper GIT, a number of mesalamine preparations (*e.g.*, delayed-release, extended-release, and enema) based on different drug delivery mechanisms such as azo-bonded prodrugs, pH-dependent, time-dependent, and multi-matrix systems are prepared, however, with certain limitations (Ye and van Langenberg, 2015). Mesalamine has low solubility and low permeability which necessitates an increase in dissolution and absorption rates to enhance its bioavailability (Tamura et al., 2020). The development of colon-specific delivery systems can reduce side effects and enhance therapeutic efficacy. Such a controlled drug delivery system (*e.g.*, microflora-activated drug release system) has the capability to impede drug release in the upper GIT and deliver the active drug to the site of inflammation in an effective and selective manner (Bayan and Bayan, 2020). This results in decreased dosing frequency and side effects, improved therapeutic efficacy, and prevention of drug degradation. Furthermore, the granule formulation of mesalamine has shown better outcomes in IBD in previous studies (Tamura et al., 2020; Ye and van Langenberg, 2015). Hence, in this study, we targeted 2,4,6-trinitrobenzenesulfonic acid (TNBS) induced experimental UC by using a microflora-activated drug release mechanism for oral colon-targeted drug delivery of mesalamine (M) delayed-release granules (DRGs) *via* polysaccharides as carriers *viz.* chitosan (C), pectin (P), and a mixture of both (PC), and evaluated their anti-oxidant and anti-inflammatory activities in the colon.

2. Materials and methods

2.1. Drugs and reagents

Mesalamine (Ind-Swift Ltd.); ethylenediaminetetraacetic acid (EDTA) and chitosan (Loba Chemie Pvt. Ltd., Mumbai), pectin (S.D Fine Chem, Limited, Mumbai); microcrystalline cellulose (Indian Research Product, Mumbai); HPMC E15LV (Oxford diagnostics, Mumbai); PVP K30, magnesium stearate, sodium cyanide, sodium carbonate, and talc (Sisco Research Laboratory, Mumbai), carboxy methyl cellulose (S.D. Pharmaceuticals), ethanol (Indian Chemical Company, Delhi); sodium nitroprusside, sodium chloride, Na_2HPO_4 (dibasic) sodium phosphate, NaH_2PO_4 (monobasic) sodium phosphate (A.B. Enterprises, Mumbai); glutathione, formalin, thiobarbituric acid (TBA), and dimethyl sulfoxide (DMSO) (Hi-Media Lab Pvt. Ltd); TNBS (2,4,6-trinitrobenzenesulfonic acid) (Sigma Aldrich, Mumbai) were used. All other chemicals and reagents were of A.R. grade.

2.2. Preparation and characterization of mesalamine delayed-release granules (DRGs)

2.2.1. Preparation of granules

Granules of mesalamine composed of various polysaccharides were synthesized using the wet granulation technique (Pawar and Ahirrao, 2014; Sharma et al., 2012; Sudarshan et al., 2009). The granules were prepared using a three-stage process. Initial preparations included combining pure mesalamine with a number of other polysaccharides, including chitosan and pectin. Three different formulations were tested for their efficacy against UC in Wistar rats. Chitosan-based mesalamine (CM) delayed-release granules (DRG) were created in the first formulation (CM-DRG). The second formulation included making mesalamine delayed-release granules out of pectin (PM-DRG). Pectin and chitosan, both were employed to make mesalamine delayed-release granules in the third formulation (PCM-DRG). In the first step, mesalamine and the other ingredients in Table 1 were thoroughly combined using the mortar and pestle. The 10 % solution of Eugragit E 100/PVP K30 was used to prepare the damp mass, and either water or isopropyl alcohol (appropriate solvent) was utilized throughout the granulating process in the case of Eudragit E 100. After everything was well combined, we sieved it through a No. 16 mesh. After collecting the wet granules, they were dried in a tray drier for 1.5 h at an input temperature of 60 ℃. The next step required collecting the dry granules and sealing them in an airtight container. The second process included slamming the granules around in a tablet compression machine. The granules used for filling the capsules were obtained by passing the collected slugs through sieves of size #60. In the past, capsules could be filled quickly and easily with dry granules

Table 1
Different polysaccharide polymer-based mesalamine delayed-release granules (DRGs) formulation.

Ingredients (mg)	Chitosan-mesalamine DRGs	Pectin-mesalamine DRGs	Pectin-chitosan-mesalamine DRGs
Mesalamine	50 mg	50 mg	50 mg
Chitosan	13.5 mg	–	9.5 mg
Pectin	–	19.5 mg	15 mg
Microcrystalline Cellulose	25.75 mg	19.75 mg	21 mg
Eudragit E 100/PVP K 30	2.5 mg	2.5 mg	2.5 mg
HPMC E15LV	6.25 mg	6.25 mg	–
Magnesium Stearate	1 mg	1 mg	1 mg
Talc	1 mg	1 mg	1 mg
Total weight (mg)	100 mg	100 mg	100 mg

after being greased with magnesium stearate and talc. Table 1 elaborates on the amounts used to make the various formulations (CM-DRG, PM-DRG, and PCM-DRG). Each of the formulations was made by combining separate polysaccharides of chitosan and pectin, whereas PCM-DRG was made by combining both polysaccharides. Several tests were performed on the finished granules to see how effective they were.

2.2.2. Calibration of suitable dilutions

Mesalamine was diluted to various concentrations (2 μg/mL, 4 μg/mL, 6 μg/mL, 8 μg/mL, and 10 μg/mL) in 0.1 N HCl and in different buffer solutions (pH 1.4, 6.0, and 7.2). Then, using a double-beam UV–visible spectrophotometer (CyberLab Analytical Instruments), the absorbance of these solutions was measured against a blank at a wavelength of 330 nm for a pH of 1.4 and 331 nm for other range of buffer solutions. A graph was plotted by taking the concentration on the x-axis and absorbance on the y-axis to get a standard curve. Mesalamine absorbance and concentration were determined.

2.2.3. Compatibility study

The compatibility test was performed before the development of dosage forms. Mesalamine was analyzed using infrared spectroscopy. Both the pure drug and the drug combined with polymers were subjected to Fourier Transform Infrared Spectroscopy (FTIR) analyses (Perkin Elmer, USA) with a scanning range of 4000–400 cm^{-1}. FTIR spectra of both drug raw material and drug with different excipients utilized in the formulation were obtained. Hydrostatic pressing at a force of 5 cm^{-2} and a resolution of 4 cm^{-1} was used to produce samples in KBr discs (2 mg sample in 200 mg KBr). To ensure the reliability of the results, the experiments were replicated thrice (Thakur et al., 2016; Arora et al., 2011).

2.2.4. Micromeritics properties of granules

The density was measured both loosely and after being tapped, thus the terms LBD (loose bulk density) and TBD (tapped bulk density) (Thakur et al., 2016; Arora et al., 2011). Each formula's granules were weighed precisely and then added to a 25 mL measuring cylinder, where it was shaken to disperse any clumps. After measuring the starting volume, the cylinder was dropped from a height of 2.5 cm onto a hard surface at 2 s intervals. We kept recording until we saw no more volume fluctuations.

LBD = Weight of the granules (g) / Volume of the packing (mL).

TBD = Weight of the granules (g) / Tapped volume of the packing (mL).

The compressibility index and Hausner's ratio were calculated as follows:

$$\%Compressibility(Carr's\ index) = [(TBD - LBD) \times 100]/Tapped\ density$$

Hausner's Ratio = TBD / LBD.

The angle of repose was determined by the funnel method. Accurately weighed granules were taken in a funnel and allowed to flow through the funnel freely onto the surface. The diameter of the cone was measured and the angle of repose was calculated using the following equation.

The angle of repose (θ) = $\tan^{-1}$ (h/r), where, h = height and r = the radius of the cone.

2.2.5. Morphology of granules

Particle-size analysis of samples ($n = 3$) was carried out using a sieve shaker using five standard US sieves of the size range of 75–1500 μm. The morphology of the samples was examined using a scanning electron microscope (SEM) and image analysis was performed (Rodriguez et al., 2002).

2.2.6. Estimation of mesalamine in granules

For the analysis of mesalamine in the dosage form, equivalent to twenty capsules of mesalamine (400 mg) were pulverized into fine particles that were thoroughly mixed. An amount containing 10 mg of the drug was taken in a volumetric flask (150 mL), ultrasonicated (45 min) in 0.1 N HCl (40 mL), and filtered using the No. 41 Whatman filter paper. Subsequently, the filter paper was treated with 0.1 N HCl and the washings were included in the filtrate thereby making the ultimate volume up to 100 mL. This solution was suitably diluted and the absorbance range of the final sample equivalent to 10 μg/mL was noted in contrast to the blank (*i.e.*, 0.1 N HCl) using a spectrophotometer.

2.2.7. Drug content uniformity

The content uniformity test is employed to make sure that each formulation has the appropriate quantity of medicinal components with little variance among capsules throughout a given batch. A typical sample of 30 capsules is chosen for the content homogeneity examination from each batch, and 10 of those samples are randomly tested. The drug (equivalent to 40 mg) from each formulation taken in a volumetric flask was ultrasonicated, and filtered (Whatman No 41), and the volume was made up to 100 mL. After dilution, each sample was analyzed spectrophotometrically. None should exceed ± 25 % of the specified potency, and a minimum of 9 should assay inside the specified limits *i.e.*, ± 15 %.

2.2.8. In-vitro drug release studies

There are different dissolution procedures for extended-release mesalamine capsules (pH 7.5 only) and delayed-release capsules (pH 1.4, 6.0, and 7.2) in the USP. However, the true residence period in diverse regions of the GIT is not adequately considered in these methods. The current technique eliminates the prerequisite of the removal of capsules or drug beads from dissolution vessels to change media (Chuong et al., 2008). It shows GIT transit times, which are absent in the USP method for mesalamine capsules. The present technique permits the comparison of different formulations. USP dissolution apparatus II was used in this method. The 1st step was in gastric fluid constituting the "acidic dissolution stage" (500 mL of 2 g/L NaCl, pH adjusted to 1.4 with HCl) for 2 h. At the end of the acidic dissolving phase, samples were taken. In 2nd step (buffer stage I), which constitutes simulation of "small intestinal fluid", 245 mL of 0.09 M sodium orthophosphate hydrate ($Na_3PO_4{\cdot}12\ H_2O$) was included to enhance the pH of the dissolution medium from 1.4 to 6.0. Dissolution was sustained for two or four hours at 100 rpm. In 3rd step (buffer stage II), which comprised a simulation of large intestinal fluid, the pH of the dissolution medium was further raised up to pH = 7.3 by the addition of 100 mL of 0.3 M sodium hydroxide (NaOH) or 87 mL of 0.05 M $Na_3PO_4{\cdot}12\ H_2O$ for further 3.5 h of dissolution testing. Thus, throughout the test, the capsule beads were not required to leave the dissolution vessel. The entire dissolution period was 5 to 7.5 h. For the 7.5 h, in the sampling schedule, the sampling times are 2 h, 3.5 h, 4.5 h, 5.5 h, 6.5 h, and 7 h. Post-dissolution investigation, the concentrations were computed by employing a UV spectrophotometer. The cumulative quantity of drug release at individual sampling time was calculated for the delayed-release granules or capsules and the cumulative % release *vs.* time graph was developed.

2.2.9. Procedure for preparation of rat cecal contents medium dissolution

By conducting the drug discharge investigation in a medium comprising rat cecal material, the sensitivity of the enzymatic activity of the large bowel bacteria on matrix capsules was evaluated (Chuong et al., 2008). Rat cecal materials were collected from five Wistar rats weighing between 240 and 300 g for the investigation. Standard food was given to animals, but for 7 days, 1 mL of pectin 2 % w/v dispersion was administered orally to stimulate the production of digestive enzymes. A Teflon cannula that may be put into the stomach was used to inject the pectin dispersion. Rats were euthanized and their abdominal cavities were dissected to retrieve their cecal materials around 45 min prior to the drug release trials. CO_2 was added to the media after the addition of the cecal contents in the phosphate buffer solution that was

previously chosen for colonic research. Each animal's cecal materials were removed and weighed individually. Five Wistar rats' cecal materials were combined to create the aggregate final 4 % w/v cecal working dilution. After the fifth hour of the trial, the 4 % cecal materials were added to the dissolving media, where they were observed for up to 20 h, or until 95 % of the drug release was achieved. The samples were taken at scheduled times regularly and subjected to UV spectrophotometer analysis. To maintain sink conditions, the withdrawn sample was substituted with a completely fresh medium that was added with carbon dioxide. For the purpose of separating the solid ingredients, the removed samples were diluted and centrifuged (CPR-30 REMI Compufuge, India). The bacteria-proof filter was used to gather the supernatant liquid, and the filtrate was then examined for the presence of mesalamine using a double-beam UV spectrophotometer with a wavelength of 330–332 nm.

2.2.10. Reproducibility dissolution

The capsules from the chosen batch were produced twice more to test the process's repeatability. The batch size was raised from 30 to 60 capsules, nevertheless. As the other criteria had been demonstrated to be repeatable and similar to the capsules from the previous batch, the capsules were tested for dissolving.

2.3. Experimental animals

The animal protocol was approved by the Institution Animals Ethics Committee (IAEC) (No. SSP/IAEC/17/03). 240–300 g male Wistar rats (adults) were procured from Venus Remedies, Baddi (India) and housed in the Animal House Facility (temperature 23 ± 2 °C, humidity 40 ± 10 %, and light–dark cycle 12 h each) within the institute. All the experiments and animal care were in accordance with the regulations set forth by the Committee for the Control and Supervision of Experiments on Animals (CCSEA), the Government of India, and ARRIVE guidelines were followed. The animals were housed in polypropylene cages and provided with a standard rodent diet (*M/s* Ashirwad Industry, Mohali, India) and purified water *ad libitum*. The animal caretakers were blinded to different treatments. Experiments were initiated after 14 days of acclimatization period and conducted between 900 and 1700 h.

2.4. Induction of ulcerative colitis (UC)

Rats were fasted overnight and anesthetized using ketamine (90 mg/kg) and xylazine (10 mg/kg) administered intraperitoneally (*i.p.*). The colon lumen was reached by inserting a polyethylene cannula with a 4 mm diameter *via* the anus and advancing it such that its tip was 6–8 cm proximal to the anus. It used to take 2 mL of normal saline and abdominal palpation to complete the lavage procedure. The 5 % w/v TNBS was diluted in an equal volume of 100 % ethanol (working solution of 2.5 % TNBS in 50 % ethanol) and given to the rats at a dose of 100 mg/kg of body weight (Xu et al., 2017). To avoid any leakage after colon treatment, the rats were held in the Trendelenburg position with their heads down for 60 s. Stool consistency and blood occult were noted. The vehicle control group was given an equal amount of 50 % ethanol devoid of TNBS.

2.5. Drug treatment schedule

On day 1 animals were randomly distributed in a single-blind pattern into 2 groups: Vehicle control (n = 5) and UC (n = 25). TNBS was administered to rats of the UC group and subsequently, animals were again randomly divided in a single-blind pattern into 5 groups (n = 5): the TNBS group was given TNBS alone; the TNBS + M group was given TNBS, and mesalamine (M); TNBS + CM-DRG was given TNBS and chitosan-mesalamine (CM) delayed-release granules (DRG); TNBS + PM-DRG was given TNBS and pectin-mesalamine (PM) delayed-release granules (DRG); and TNBS + PCM-DRG was administered TNBS and pectin-chitosan-mesalamine (PCM) delayed-release granules (DRG). Mesalamine and three different polymer-based mesalamine delayed-release granules were given in doses of 50 mg/kg using 0.5 % w/v sodium carboxymethylcellulose (dosing volume 10 mL/kg) vehicle *via* oral gavage for 14 days daily (Shibrya et al., 2023). The dosing was initiated 24 h after intracolonic TNBS instillation (Fig. 1). The vehicle control and TNBS groups were given an equal amount of 0.5 % w/v sodium carboxymethylcellulose vehicle. The body weight (g) of experimental animals was measured and analyzed weekly.

2.6. Collection of samples and preparation

On day 14, blood was extracted from the *retro*-orbital plexus (under anesthesia) in EDTA-added (anticoagulant 2 mg/mL) tubes. HumaStar-300 (clinical autoanalyzer) was used to determine the white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (HGB), hematocrit (HCT) %, and platelet count (PLT) in a blood sample. After blood collection, all animals were euthanized 24 h after the last dose. Each animal's colon was dissected to measure length and weight. Colon tissues were homogenized (10 % w/v) in ice-cold 0.1 M phosphate buffer solution (pH 7.4) in a 9:1 ratio with protease inhibitors leupeptin (4 μg/μL), pepstatin A (4 μg/μL), aprotinin (5 μg/μL), and phenylmethylsulfonyl fluoride (0.2 mM) and centrifuged at 3500 × *g* for 15 min at 4 °C. The supernatant was separated and immediately stored at −80℃ till further analysis. Parameters of oxidative stress such as malondialdehyde (MDA) (Triantafillidis et al., 2014), glutathione (GSH) (Ellman, 1959), superoxide dismutase (SOD) activity (Suzuki, 2000), and total nitrites (Sastry et al., 2002) were measured. Enzyme-linked immunosorbent assay (ELISA reader, Biorad) was used to quantify myeloperoxidase (MPO) activity and the expression of nuclear factor erythroid 2-related factor 2 (Nrf2) and inflammatory (interleukin-1β) cytokines in colon lysate as per the standard procedure mentioned in the kits (Krishgen Biosystems, India).

2.7. Disease activity index (DAI)

The clinical severity of colitis was evaluated by observing the body weight, stool consistency, and presence/absence of blood occults in the rectum (Wirtz et al., 2017). Each of these parameters was assigned a score based on the criteria (Table 2). The mean of these parameters was used to calculate DAI.

DAI is the combined scores of weight loss, stool consistency, and bleeding divided by 3.

2.8. Colon macroscopic damage evaluation

Cervical dislocation under anesthesia technique (sodium pentobarbitone, 150 mg/kg, *i.p.*) was used to euthanize the rats. A 10 cm long section of the colon's distal end was removed, sliced lengthwise, rinsed in ice-cold water, and then phosphate-buffered saline to flush out the feces, dried, and evaluated. Together, the ulcer score and ulcer area (mm^2) made up the ulcer index (UI). Two separate blind observers performed the semi-quantitative grading. Damage to the colon was graded on a scale from 0 to 5, with 0 indicating no change, no ulcer, and no inflammation; 1 indicating local hyperemia but no ulcers; 2 indicating an ulcer without hyperemia and bowel wall thickening; 3 indicating ulceration and inflammation at a single site; 4 indicating ulceration at two or more sites; and 5 indicating ulceration that extended more than 2 cm.

2.9. Histopathology

The section of the colon that was removed was preserved at 4℃ in 10 % formalin with a saline phosphate buffer (with 0.05 % sodium azide). After a week, the samples were subjected to a sequential clearing and dehydrating procedure, and paraffin blocks were prepared. Colon tissues were dissected into 6 μm slices and stained with hematoxylin and eosin

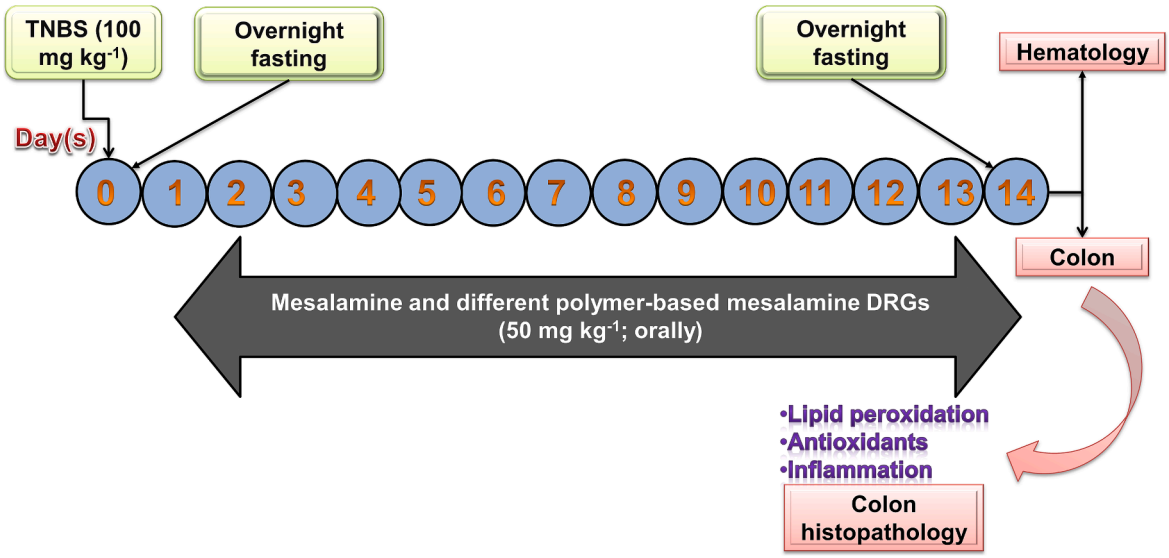

Fig. 1. Experimental protocol.

Table 2
Disease activity index scoring.

Weight loss (%)	Stool consistency	Blood occults	Score
Normal	Normal	Normal	0
1–5	Soft		1
5–10	Loose	Hemoccult + ve	2
10–20			3
> 20	Watery diarrhoea	Gross bleeding	4

(H&E). The slides were examined using a Polarized Optical Microscope (POM, Nikon Eclipse LV100POL, Japan) at × 400 magnification. Mucosal damage, necrosis (n), crypt hyperplasia (ch), leukocyte infiltration (li), and edema (e) were noted by a blinded observer, and the histopathological score was determined using previously mentioned microscopic evaluation criteria (Khairy et al., 2018).

2.10. Statistical analysis

Data was collected and analyzed by an experienced researcher blinded to various drug treatments. The data were statistically analyzed using GraphPad Prism 8.0. The data was normally distributed and there were no outliers. One- or repeated measures two-way ANOVA and Dunnett's or Bonferroni multiple comparison tests were used to analyze the data and determine the differences between the treatment groups. Data are presented as mean ± standard deviation (S.D.)/standard error of the mean (S.E.M.). A value of $p < 0.05$ is considered significant.

3. Results

3.1. Calibration and compatibility studies

The standard curve was used for the estimation of mesalamine in the present study (Fig. 2). Both the pure mesalamine and its combination with polymers were subjected to Fourier-transform infrared (FTIR) spectroscopy analyses. Table 3 lists the most prominent sharp and important peaks (functional groups) in the spectra of the drug and the drug and polymer combination (Fig. 3). According to the FTIR interpretation, there was no evidence of interaction between the medication and the polymer. Mesalamine drug spectra did not display the C = O stretch value of the carboxylic acid group.

3.2. Morphology and characterization of different polysaccharide-based mesalamine DRGs

The chitosan-mesalamine (CM) DRGs showed the highest value of flow characteristics (22.08 ± 0.05), and the loose bulk density of different formulations varied from 0.422 ± 0.003 to 0.434 ± 0.002. Bulk density measured from core samples varied from 0.517 ± 0.003 to 0.533 ± 0.020. Hasuner's ratio for all formulations is close to 1, while Carr's index for all compounds varied from 12.67 ± 0.2 to 21.95 ± 2.7. The angle of repose (θ) and Hausner's ratio of the formulation depicted satisfactory flow properties (Table 4).

The granule size analysis indicated an irregular distribution. Each different polysaccharide-based DRG has its own prevalent fraction: CM-DRG 105–500 μm, PM-DRG 75–1000 μm, PCM-DRG 75–1500 μm. The 250 μm size was used for imaging studies. The morphology of the granules is shown in Fig. 4. The comparison between different DRGs indicated that CM-DRG has a more spherical shape and the surface is quite irregular. PM-DRG and PCM-DRG have irregular shapes. Image analysis was performed in order to better analyze the information provided by SEM (Table 5). CM-DRG has a greater surface area in comparison to the other two formulations. Furthermore, the AR value of CM-DRG is low and SF is high relative to PM- and PCM-DRG which indicates more spherical-shaped granules.

3.3. In-vitro drug release studies

Drug content uniformity and *in-vitro* drug release research were conducted on the formulated granules (Fig. 5). Granule's conformity to the pharmacopeial standards for homogeneity of drug content was satisfactory. Ten different formulations were tested for content uniformity using assay limits, nine of them were found to be within 15 % of their reported potency and none were found to be over 25 %. *In-vitro* drug release studies were performed on the formulated granules in buffers with pH ranges that imitated the gastric and intestinal tract. Therefore, tests of drug release were performed *in-vitro* for 7.5 h at various pH levels (1.4, 6, and 7.2) to mimic the stomach environment. All of the tested formulations showed a delayed-release pattern in the pharmacological tests. After 2 h, the CM-DRGs formulation released 10.75 % of the medicine, the PM-DRGs formulation released 19.92 %, and the PCM-DRGs formulation released 26.52 % of the drug. To the greatest extent possible, the medication was released from the PM formulation; this formulation accounts for around 95 % of the drug's total release. All formulations, with the exception of CM-DRGs, exhibit a

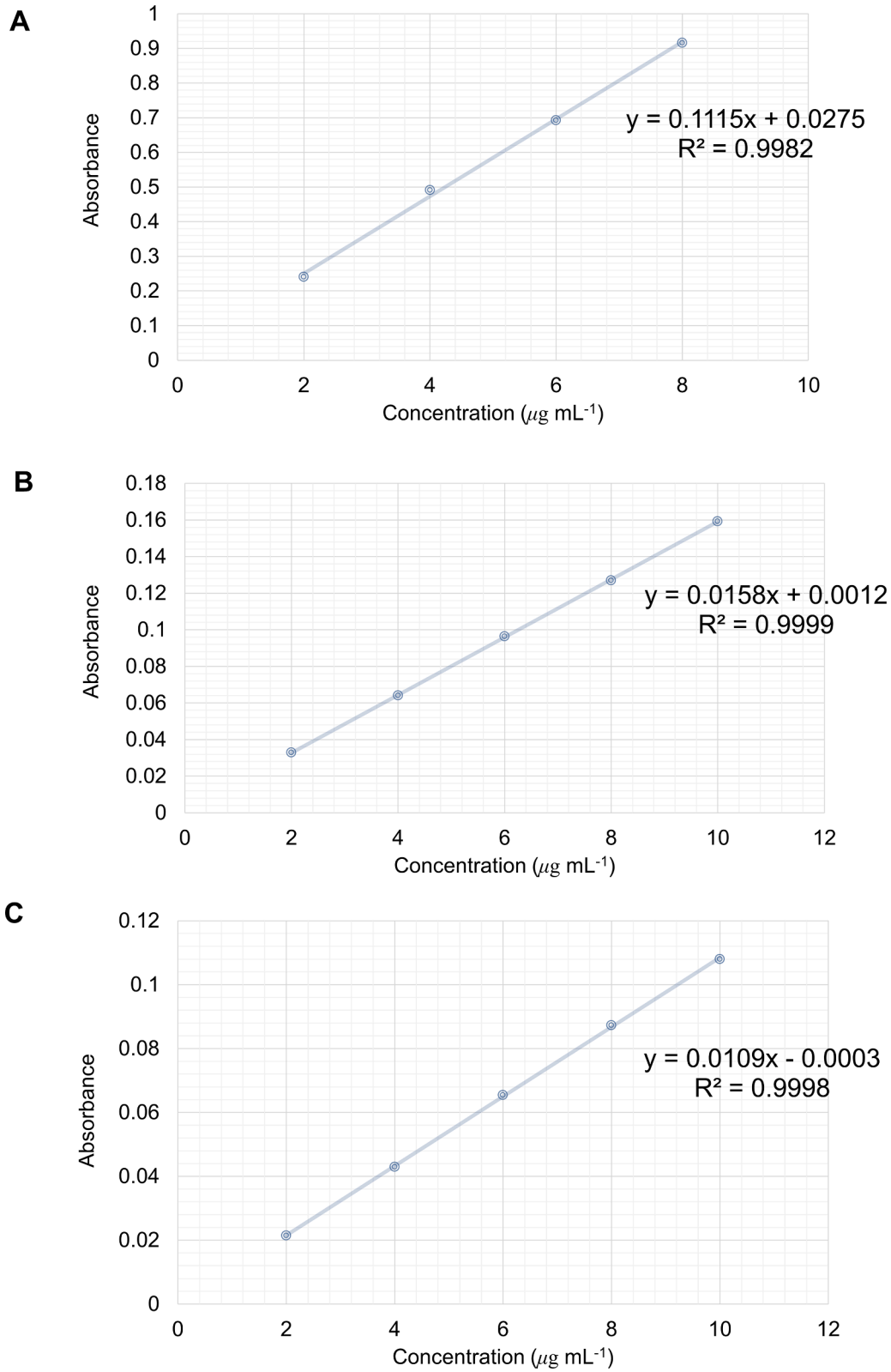

Fig. 2. Standard curve for (A) pH 1.4, (B) pH 6.0, and (C) pH 7.2.

clearly defined or significantly delayed release profile after 5.5 h of dissolving testing. According to the results of the dissolving trial, the formulation that included chitosan (CM-DRGs) for drug delivery was found better with respect to the rest of the formulations.

3.4. Effect of mesalamine, CM-, PM-, and PCM-DRGs on body weight, colon metrics, ulcer index (UI), and diarrhea against TNBS-induced UC in rats

There was no significant change in the body weight of rats of different groups during the 1st week. However, a significant decline in

Table 3
FTIR drug-excipient interaction study summary.

Functional groups	Stretching/ bending or Deformation	Pure mesalamine (cm^{-1})	Mesalamine + pectin + other excipients (cm^{-1})	Mesalamine + chitosan + other excipients (cm^{-1})
Aromatic ring	i) C–H $_{stretch}$	3084.12	3085.23	NA
	ii) C = C $_{stretch}$	1654.17	1650.09	1652.17
		1620.13	1620.78	1621.56
Carboxylic acid group	i) O–H $_{stretch}$	2778.50	2787.42	2785.81
	ii) C = O $_{stretch}$	NA	1795.14	1795.10
Hydroxy group	i) C-O $_{stretch}$	1242.85	1242.92	1242.98
Amine	i) C-N $_{stretch}$	1266.04	1266.24	1266.20
		1314.68	1314.66	1314.70

mean body weight was observed after 2nd week *i.e.*, on day 14 in the rats of the TNBS group relative to the control rats, and this decline was remarkably attenuated by mesalamine (50 mg/kg) and different polymer-based mesalamine DRGs (50 mg/kg). TNBS (100 mg/kg) significantly ($p < 0.05$) enhanced the colon weight/length ratio, UI, and diarrhea score in rats relative to the control group rats. We also observed blood occult in the watery stools of rats that were treated with TNBS. Administration of mesalamine and different polymer-based mesalamine DRGs for 14 days daily attenuated the TNBS-induced increase in colon weight/length ratio, UI, and diarrhea score in rats. However, CM-DRGs and PCM-DRGs caused a significant ($p < 0.05$) amelioration of body

Table 4
Flow and packing properties.

Preparation	Angle of repose (θ)	LBD (g/ cm^3)	TBD (g/ cm^3)	Carr's index (%)	Hasuner's Ratio
CM-DRG	22.08 ± 0.05	0.434 ± 0.002	0.517 ± 0.003	12.67 ± 0.2	1.10
PM-DRG	25.9 ± 0.04	0.422 ± 0.003	0.520 ± 0.001	14.22 ± 0.5	1.08
PCM-DRG	27.57 ± 0.03	0.423 ± 0.003	0.533 ± 0.020	21.95 ± 2.7	1.24

Data are presented as Mean ± S.D. LBD: Loose bulk density, TBD: Tapped bulk density, CM-DRG: Chitosan-mesalamine delayed-release granules, PM-DRG: Pectin-mesalamine delayed-release granules, PCM-DRG: Pectin-chitosan-mesalamine delayed-release granules.

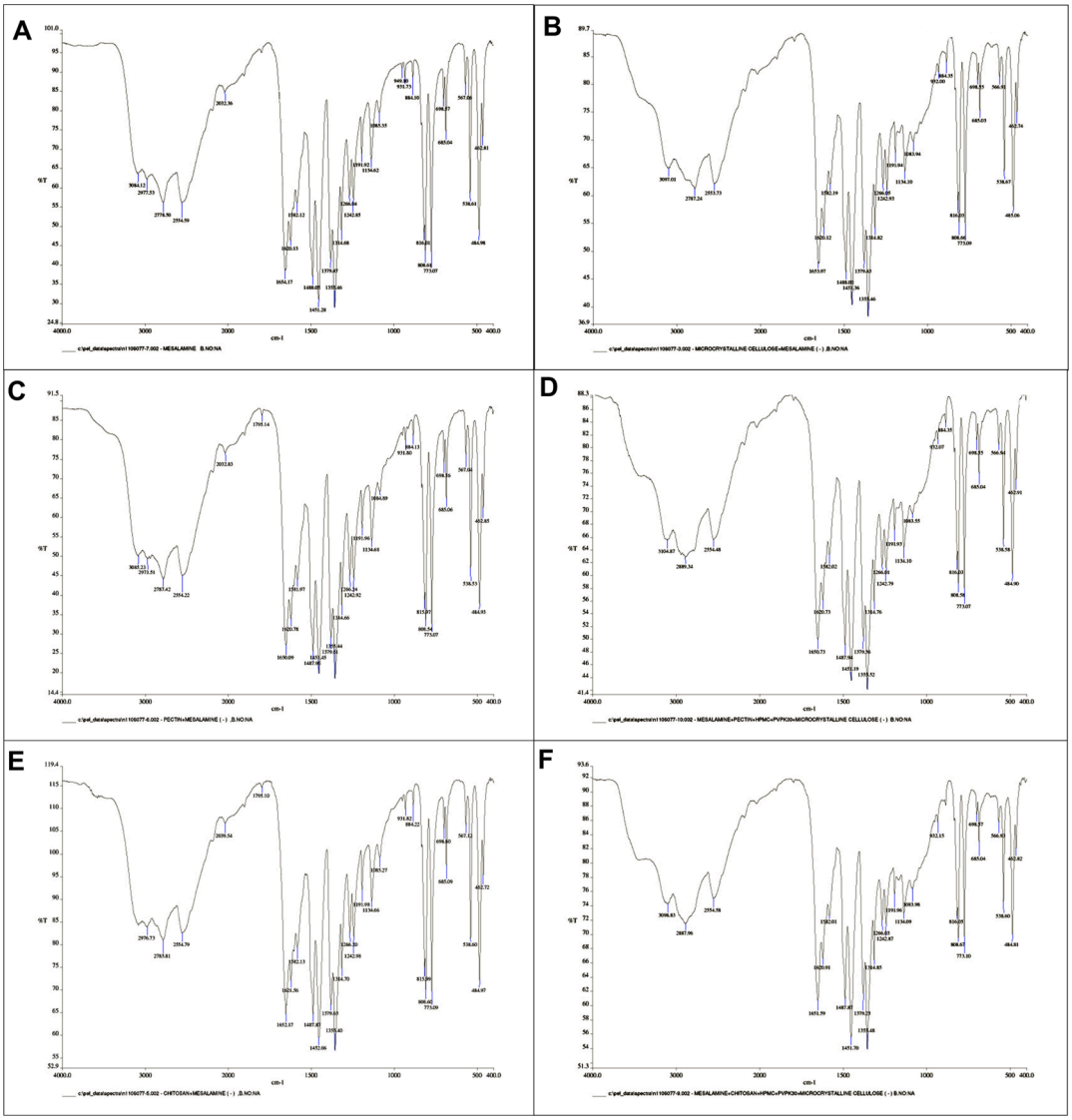

Fig. 3. FTIR spectrum – (A) mesalamine pure drug, (B) microcrystalline cellulose-mesalamine, (C) pectin-mesalamine mixture, (D) pectin-mesalamine, and other ingredients, (E) chitosan-mesalamine mixture, (F) chitosan-mesalamine, and other ingredients.

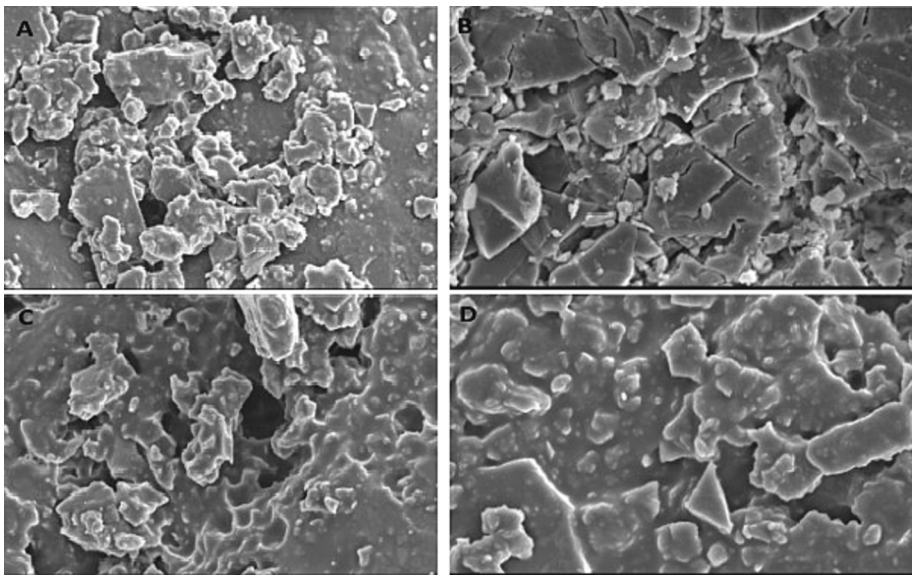

Fig. 4. SEM images of (A) Mesalamine pure drug, (B) CM-DRG, (C) PM-DRG, and (D) PCM-DRG.

Table 5
Image analysis- Size and shape of DRGs.

Sample	Area ± SD (mm^2)	Aspect ratio ± SD (AR)	Shape factor ± SD (SF)
CM-DRG	23.782 ± 3.998	2.01 ± 0.726	0.591 ± 0.017
PM-DRG	20.161 ± 2.983	2.16 ± 0.673	0.441 ± 0.022
PCM-DRG	20.221 ± 3.563	2.28 ± 0.837	0.399 ± 0.008

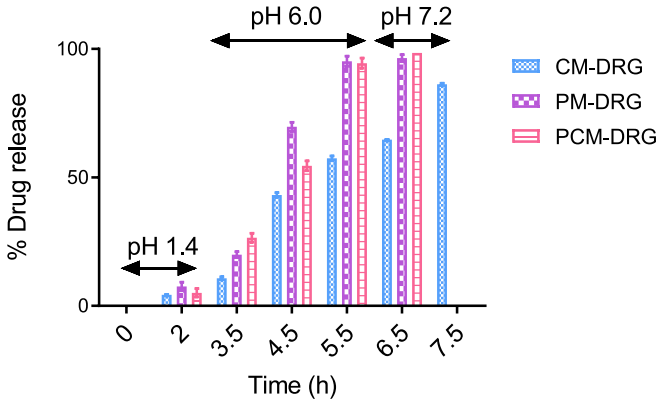

Fig. 5. Comparative *in-vitro* drug release profile of the experimental batches. Data are presented as Mean ± S.D. CM-DRG: Chitosan-mesalamine delayed-release granules formulation, PM-DRG: Pectin-mesalamine delayed-release granules formulation, PCM-DRG: Pectin-chitosan-mesalamine delayed-release granules formulation.

weight, colon profile, and UI, and significantly arrested diarrhea in TNBS-treated rats relative to TNBS and vehicle administration. Data indicated that treatment with CM-DRGs caused more pronounced amelioration of body weight, colon weight/length ratio, UI, and diarrhea score in TNBS-induced rats relative to treatment with mesalamine, PM-DRGs, and PCM-DRGs (Fig. 6).

3.5. Effect of mesalamine, CM-, PM-, and PCM-DRGs on DAI against TNBS-induced UC in rats

The DAI of TNBS-treated animals was significantly ($p < 0.05$) higher relative to the vehicle treatment in control rats. Mesalamine (50 mg/kg) caused a moderate ($p > 0.05$) decrease in TNBS-induced rise in DAI in comparison to the TNBS and vehicle treatment in rats (Fig. 7). CM-, PM-, and PCM-DRGs (50 mg/kg) caused a significant ($p < 0.05$) decrease in DAI in TNBS-treated Wistar rats relative to the TNBS and vehicle-treated rats. CM-DRGs caused a more pronounced decline in DAI in TNBS-induced rats relative to treatment with mesalamine, PM-DRGs, and PCM-DRGs.

3.6. Effect of mesalamine, CM-, PM-, and PCM-DRGs on blood parameters against TNBS-induced UC in rats

Data revealed that TNBS substantially enhanced WBCs and platelet (PLT) count, and decreased RBCs, hemoglobin (HGB), and hematocrit (HCT)% in the blood of rats relative to the vehicle treatment in control rats. Administration of mesalamine pure drug (50 mg/kg) significantly ($p < 0.05$) attenuated TNBS-induced rise in WBCs, however, the effect on other blood parameters was not significant ($p > 0.05$) in comparison to the TNBS and vehicle treatment in rats (Fig. 8). PM-DRGs caused a significant ($p < 0.05$) decrease in WBCs and an increase in RBCs and HGB, however, its effect on other blood parameters was not significant ($p > 0.05$) in TNBS-induced Wistar rats. Administration of PCM-DRGs significantly ($p < 0.05$) attenuated WBC count and increased RBCs, HGB, and HCT% in the blood of rats relative to the TNBS and vehicle-treated rats. In contrast, CM-DRGs caused a significant ($p < 0.05$) decrease in WBCs and platelet count and increased the RBCs, HGB, and HCT% in TNBS-induced rats with respect to rats that received TNBS and vehicle only.

3.7. Effect of mesalamine, CM-, PM-, and PCM-DRGs on colonic oxido-nitrosative stress against TNBS-induced UC in rats

The biochemical analysis of colon tissue showed a substantial ($p < 0.05$) rise in malondialdehyde (MDA) and total nitrites and a decline in the reduced glutathione (GSH) levels and superoxide dismutase (SOD) activity against TNBS-induced UC in rats with respect to vehicle-treated controls (Fig. 9). Administration of mesalamine and different polymer-based mesalamine DRGs (50 mg/kg) for 14 days daily attenuated ($p < 0.05$) TNBS-induced increase in colon MDA and total nitrites and depreciation in the GSH levels and SOD activity in rats relative to the vehicle and TNBS treatment only. CM-DRG treatment showed a more pronounced decrease in oxidative and nitrosative stress and an upsurge

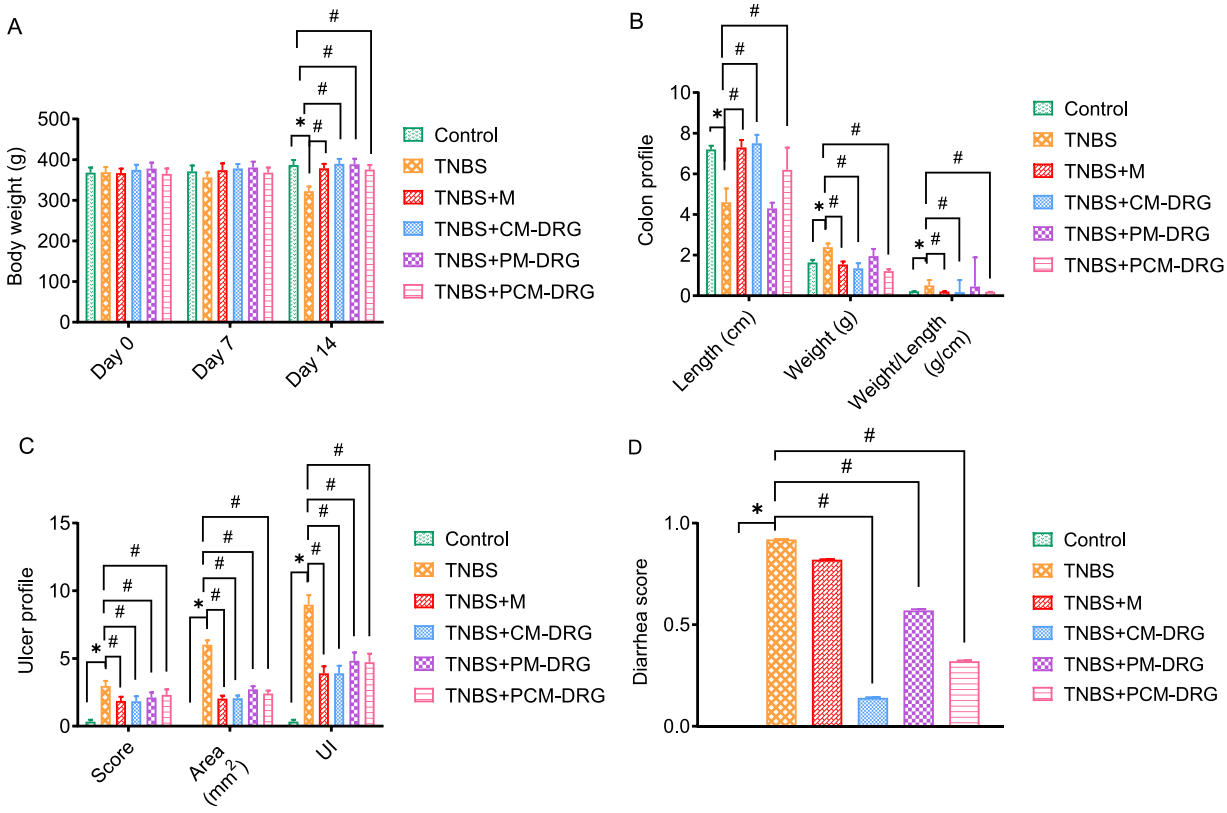

Fig. 6. Effect of mesalamine and different polymer-based mesalamine delayed-release granules (DRGs) on (A) mean body weight (g), (B) colon profile, (C) ulcer profile, and (D) diarrhea score in TNBS-induced UC in Wistar rats. Values as expressed as Mean ± S.E.M. ($n = 5$ animal/group). Data on body weight were analyzed using repeated measures two-way ANOVA and Bonferroni test. A one-way ANOVA and Dunnett's multi-comparison test were used for other data. * $p < 0.05$ *vs.* control and # $p < 0.05$ *vs.* TNBS group. M: Mesalamine, CM-DRG: Chitosan-mesalamine delayed-release granules, PM-DRG: Pectin-mesalamine delayed-release granules, PCM-DRG: Pectin-chitosan-mesalamine delayed-release granules.

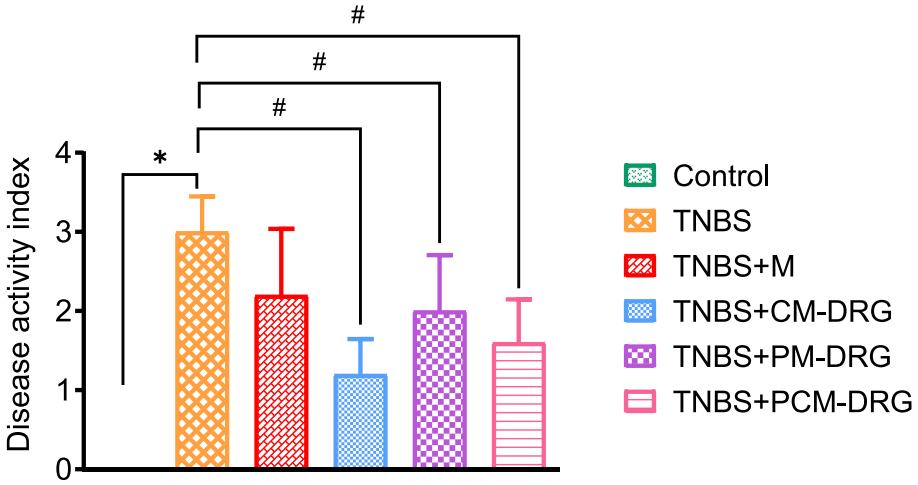

Fig. 7. Effect of mesalamine and different polymer-based mesalamine delayed-release granules (DRGs) on disease activity index (DAI) in TNBS-induced UC in Wistar rats. Values as expressed as Mean ± S.E.M. ($n = 5$ animal/group). A one-way ANOVA and Dunnett's multi-comparison test were used. * $p < 0.05$ *vs.* control and # $p < 0.05$ *vs.* TNBS group. M: Mesalamine, CM-DRG: Chitosan-mesalamine delayed-release granules, PM-DRG: Pectin-mesalamine delayed-release granules, PCM-DRG: Pectin-chitosan-mesalamine delayed-release granules.

in endogenous antioxidants in comparison to mesalamine and the other two polymer-based mesalamine DRGs against TNBS-induced UC in rats.

3.8. Effect of mesalamine, CM-, PM-, and PCM-DRGs on myeloperoxidase (MPO), nuclear factor erythroid 2-related factor 2 (Nrf2), and interleukin (IL)-1β against TNBS-induced UC in rats.

Data from ELISA indicated a substantial ($p < 0.05$) rise in colon MPO activity and IL-1β levels and a decline in the Nrf2 in TNBS-induced UC in rats with respect to vehicle-treated controls (Fig. 10). Treatment with mesalamine and different polymer-based mesalamine DRGs (50 mg/kg) for 14 days daily attenuated ($p < 0.05$) TNBS-induced increase in MPO activity and IL-1β levels and a decline in the Nrf2 in rat colon relative to the vehicle and TNBS treatment only. CM-DRG treatment displayed an additionally noticeable decrease in MPO activity and IL-1β levels and an upsurge in the Nrf2 in comparison to mesalamine and the other two

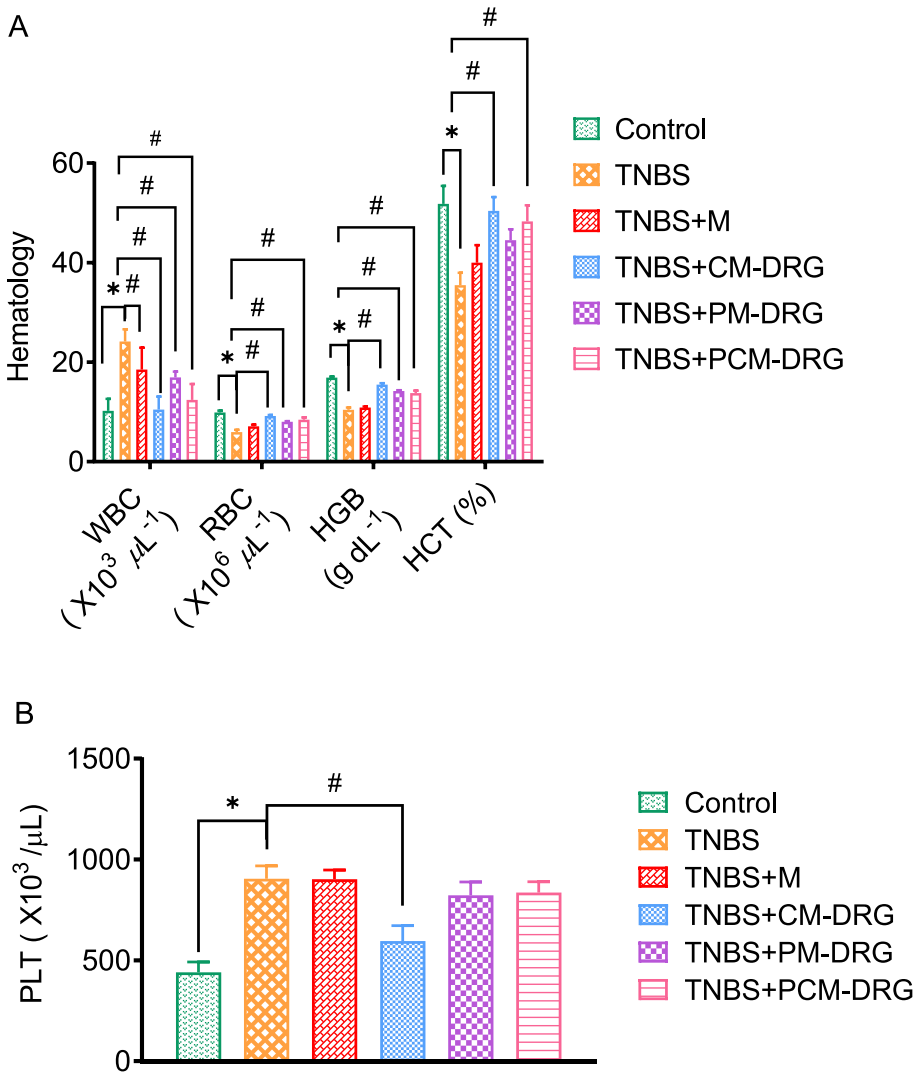

Fig. 8. Effect of mesalamine and different polymer-based mesalamine delayed-release granules (DRGs) on blood parameters in TNBS-induced UC in Wistar rats. Values as expressed as Mean ± S.E.M. ($n = 5$ animal/group). A one-way ANOVA and Dunnett's multi-comparison test were used. * $p < 0.05$ *vs.* control and # $p < 0.05$ *vs.* TNBS group. M: Mesalamine, CM-DRG: Chitosan-mesalamine delayed-release granules, PM-DRG: Pectin-mesalamine delayed-release granules, PCM-DRG: Pectin-chitosan-mesalamine delayed-release granules, PLT: Platelets, WBC: White blood cells, RBC: Red blood cells, HGB: Hemoglobin, HCT: Hematocrit. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

polymer-based mesalamine DRGs against TNBS-induced UC in rats.

3.8. Effect of mesalamine, CM-, PM-, and PCM-DRGs on colon histopathology against TNBS-induced UC in rats

The histology analysis exhibited severe and widespread colon damage by TNBS. Necrosis, leukocyte infiltration, crypt hyperplasia/abscess, and edema were the key features observed in the colon histology of TNBS-treated animals reminiscent of acute severe UC (Fig. 11). However, treatment of rats, which previously received TNBS, with mesalamine and different polymer-based mesalamine DRGs (50 mg/kg) attenuated the acute pathogenic effect of TNBS in the colon. The histology score displayed that CM-DRGs and PCM-DRGs caused a significant ($p < 0.05$) decline in colon necrosis, leukocyte infiltration, crypt hyperplasia, and edema against TNBS in rats. CM-DRGs showed a more pronounced ameliorative effect on the rat colon with respect to PCM-DRGs against TNBS.

4. Discussion

A number of mesalamine formulations in the form of delayed-release capsules and tablets *e.g.*, Mesacol® (Sun-Pharma), Cosacol® (Wallace), and Coolgut® (Torrent) are available (differentiated by dug delivery mechanisms) for use in mild-moderate UC (Ham and Moss, 2012). However, each of the present delivery mechanisms and formulations maintains the disease condition but does not limit the progress of the disease. They pose certain challenges in UC therapeutics (Sehgal et al., 2018). Hence, in this study, we utilized microflora-activated systems and prepared different polymer-based *i.e.*, chitosan (C), pectin (P), and a mixture of both (PC) mesalamine delayed-release granules (DRGs) to evaluate their efficacy in experimental UC in Wistar rats. UC requires a high concentration of drug candidates in the colon as the colon is the important area where the disease is present. There are various techniques available to treat UC by delayed-release formulation. The delayed-release formulation is developed in such a way that the formulation should release a limited amount of drug at least less than 10–20 % in the upper part of GIT so that the maximum amount of drug is delivered to the colon to treat the UC successfully. Polysaccharides are unique polymers that cannot be digested by the upper part of GIT and they remain intact until they reach the colon part. Chitosan (β-(1/4)-linked D-glucosamine and *N*-acetyl-D-glucosamine units) and pectin (1/4-linked α-D-galacturonic acid) both are natural and gastric acid-

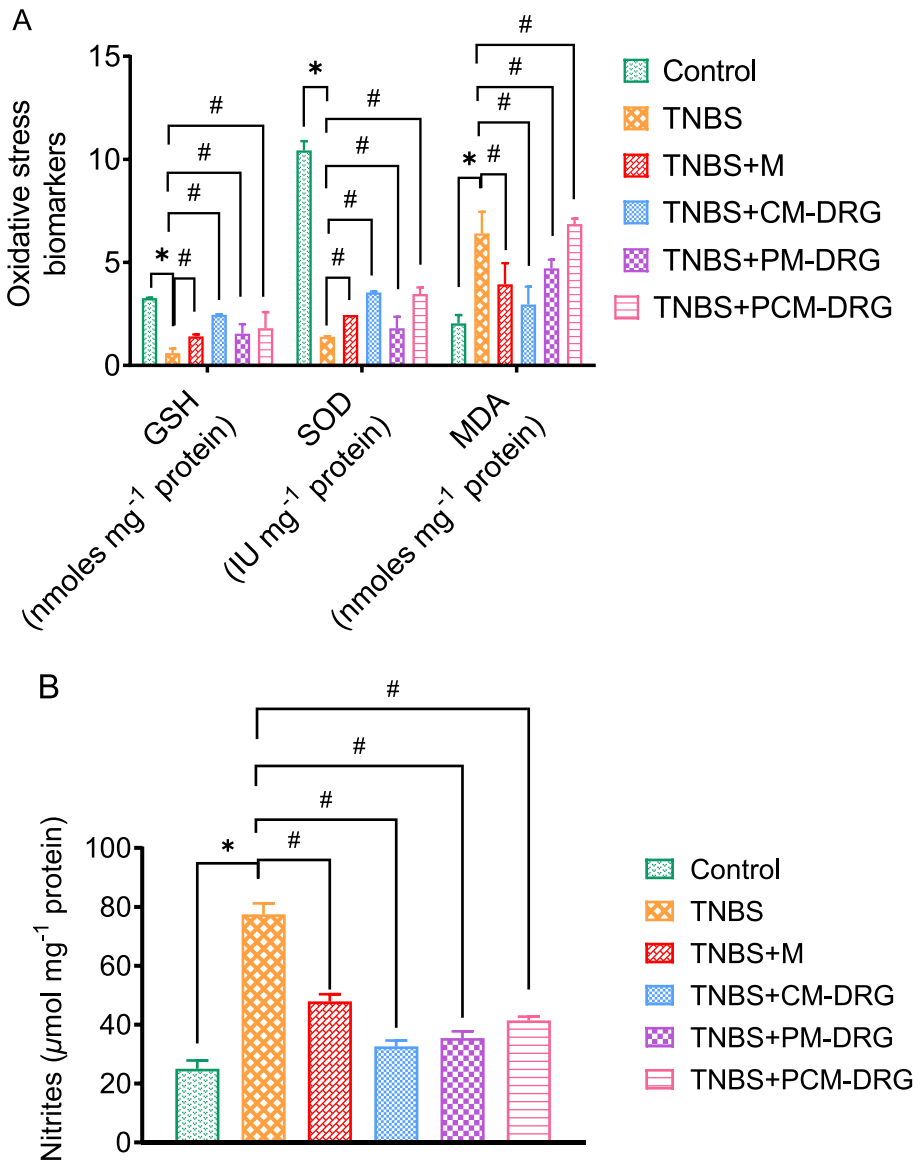

Fig. 9. Effect of mesalamine and different polymer-based mesalamine delayed-release granules (DRGs) on oxidative and nitrosative stress in the colon of TNBS-treated Wistar rats. Values as expressed as Mean ± S.E.M. ($n = 5$ animal/group). A one-way ANOVA and Dunnett's multi-comparison test were used. * $p < 0.05$ *vs.* control and # $p < 0.05$ *vs.* TNBS group. M: Mesalamine, CM-DRG: Chitosan-mesalamine delayed-release granules, PM-DRG: Pectin-mesalamine delayed-release granules, PCM-DRG: Pectin-chitosan-mesalamine delayed-release granules, GSH: Glutathione (reduced), SOD: Superoxide dismutase, MDA: Malondialdehyde.

resistant polysaccharide polymers which release drugs in the colon and have mucoadhesive properties, however, chitosan is vulnerable to very low pH and might release the drug in the stomach (Chen et al., 2022; Wong et al., 2011). Pectin is also water-soluble which may lead to early drug release. Both chitosan and pectin are non-toxic and enzymatically degradable which facilitates their use as good polysaccharide polymers in drug delivery (Deshmukh, 2020). Different concentration of chitosan and pectin was used in the three formulations as described in formulation Table 1. The granules were prepared by using the conventional wet granulation technique by using strong binder Eudragit E 100 and PVP K30 in which Eudragit E 100 is an enteric coating polymer that will enhance the delayed-release properties of the developed formulation. The low viscosity grade HPMC E15LV is used in order to control the drug release and to improve the strength of the granules for a sustained drug release profile. The granules were subjected to slugging by the tablet compression machine. The slugged granules were passed through the appropriate sieves so that the granules could be reduced into sizes that were convenient to fill in the capsules.

In this study, FTIR data of pure mesalamine and different polymer-based formulations (*i.e.*, chitosan, pectin, and a mixture of both) showed no interaction between the drug and polymer. All the excipients were compatible with the mesalamine as shown by FTIR studies. The pre-formulation tests (*e.g.*, angle of repose, Carr's index, Hausner's ratio, LBD, and TBD) showed satisfactory results indicating acceptable packing and flow properties of DRGs as per the standards. The developed formulations were subjected to drug content uniformity and *in-vitro* drug release study. The drug content uniformity of formulation was within the pharmacopeia's limits. The assay limits of content uniformity of 10 formulations were analyzed, out of which 9 formulations were within 15 % of the declared potency and none exceeding ± 25 %. The developed granules were subjected to an *in-vitro* drug release study in different pH buffers, mimicking the different pH conditions of GIT. The drug release studies were conducted as described by Chuong et al (2008) in which the removal of capsules/granules from dissolution vessels is not required for a change of media. The drug release studies revealed that the evaluated formulations followed the delayed-release pattern. The

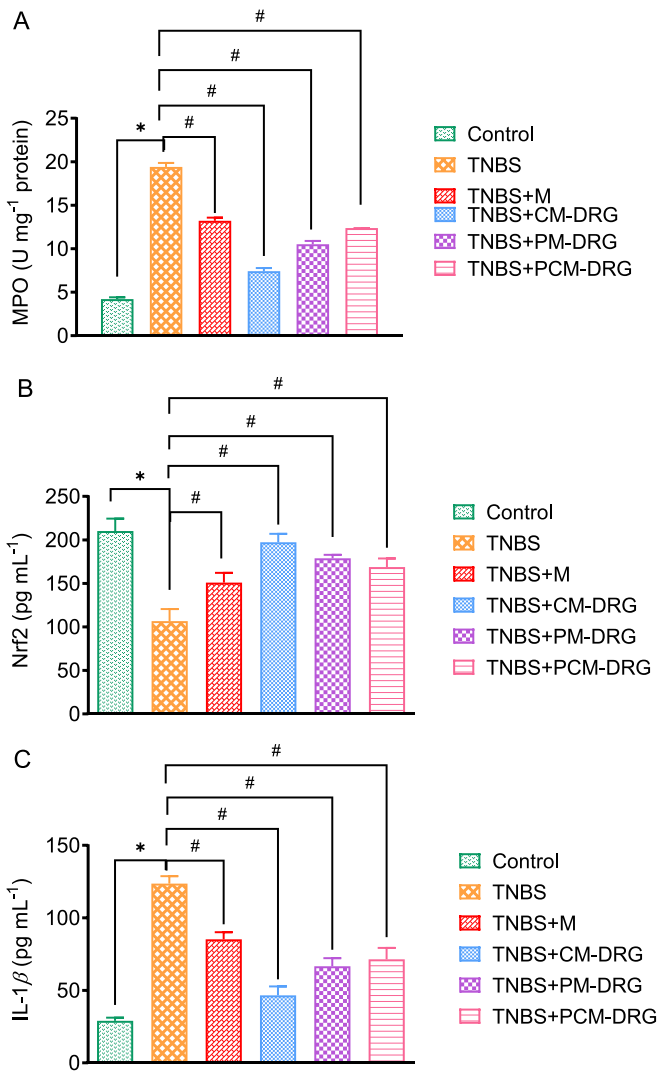

Fig. 10. Effect of mesalamine and different polymer-based mesalamine delayed-release granules (DRGs) on MPO, Nrf2, and IL-1β in the colon of TNBS-induced Wistar rats. Values as expressed as Mean ± S.E.M. (n = 5 animal/group). A one-way ANOVA and Dunnett's multi-comparison test were used. * $p < 0.05$ *vs.* control and # $p < 0.05$ *vs.* TNBS group. M: Mesalamine, CM-DRG: Chitosan-mesalamine delayed-release granules, PM-DRG: Pectin-mesalamine delayed-release granules, PCM-DRG: Pectin-chitosan-mesalamine delayed-release granules, MPO: Myeloperoxidase, IL-1β: Interleukin-1β, Nrf2: Nuclear factor erythroid 2-related factor 2.

formulation CM-DRGs, PM-DRGs, and PCM-DRGs released 10.75 %, 19.92 %, and 26.52 % of the drug at the end of 2 h of dissolution study respectively. The formulation PM-DRGs released the maximum amount of drug, ~ 95 % of the drug was released from this formulation. At the end of 5.5 h of dissolution study, amongst all the formulations CM-DRGs demonstrate a well-defined delayed-release profile as compared to the other two formulations. The drug release pattern demonstrated that the formulation prepared with chitosan-mesalamine (CM-DRGs) deserved to be declared as the best formulation as per the data of the dissolution study.

In comparison to others, the polysaccharide-based colon-targeted delivery system remains unaffected by gastric and/or intestinal milieu. This delivery mechanism is based on the bacterial count as the polysaccharides are digested by the bacteria when they reach the colon (Ibrahim, 2023). However, the efficacy of this system is limited by the reduction in bacterial count in UC. Hence, the susceptibility of the enzymatic activity of the colonic bacteria on DRG-loaded matrix capsules was assessed by performing the drug release study in a medium containing rat cecal contents. The rat cecal contents were included in the dissolution media. The result of *in-vitro* drug release demonstrated the delayed-release pattern for all three developed formulations. The in-vitro drug release studies were conducted for 7.5 h in different pH conditions such as 1.4, 6, and 7.2 to mimic the gastric condition. Data indicated satisfactory drug release via the digestion of polysaccharides by the bacteria present in rat cecal contents which confirmed the validity of this drug delivery mechanism in experimental UC.

In the current study, experimental colitis was induced by using TNBS (100 mg/kg) in an ethanol solution administered via the rectal route in the colon of rats (Brenna et al., 2013). Ethanol was used as a vehicle to disrupt the intestinal barriers (*e.g.*, mucosa) leading to exposure of the innermost layers of the colon towards haptenization by TNBS (Brenna et al., 2013). TNBS drives inflammatory mechanisms via aberrant immune activation and attenuation of mucosal repair and also activates pro-oxidant pathways leading to necrotic cell death and disruption of the intestinal mucosa (Antoniou et al., 2016). In the current experiments, TNBS caused a fall in the mean body weight of animals and caused severe acute damage in the colon which was eminent by derangement of colon profile, UI, and diarrhea. TNBS caused a severe rise in the DAI in rats. The histopathology of the colon by H&E stain also showed disruption of colon mucosa and pathogenic events in the deep layers of the colon of rats by TNBS. TNBS caused a significant rise in the histology score of rats relative to the controls. However, the oral administration of mesalamine and different polysaccharide-based mesalamine DRGs (50 mg/kg) for 14 days daily attenuated TNBS-induced depreciation in mean body weight and improved colon profile in rats. CM-, PM-, and PCM-DRGs decreased UI, DAI, and attenuated diarrhea against TNBS-induced experimental colitis in rats. The histology of the colon also indicated a decrease in mucosal damage, leukocyte infiltration, necrosis, crypt abscesses, and crypt hyperplasia in rats that were administered CM-, PM-, and PCM-DRGs after TNBS induction of UC. Data showed that oral administration of CM-DRGs significantly ameliorated mean body weight, and attenuated colon damage, ulcer area, and diarrhea. CM-DRGs significantly decreased the histology score of the colon in comparison to treatment with TNBS and vehicle only.

The hematological evaluation showed a decrease in RBCs, HGB, and HCT%, and an increase in WBC count, and PLTs in response to intracolonic instillation of TNBS in rats. Anemia is the most common extra-intestinal complication in IBD and UC (Rogler and Vavricka, 2015). Iron and vitamin B12 deficiency due to blood loss, impaired uptake, less absorption time, and low levels of serum protein/albumin are the major reasons for anemia in UC (Rogler and Vavricka, 2015; Glabska et al., 2019). An increased level of WBCs signifies underlying chronic inflammation and edema condition in the large bowel (Mack et al., 2020). Clinical data has revealed an increase in PLT count (reactive thrombocytosis) in UC (Yan et al., 2013) and is regarded as an important biomarker of this inflammatory disorder (Khairy et al., 2018). Microvascular thrombosis and thromboembolism as a result of PLT activation and PLT-WBC interaction are the key clinical features in UC. Hyperresponsive procoagulant mechanisms and PLT activation drive the inflammatory cascade in the colon (Yan et al., 2013). In this study, oral administration of mesalamine caused a significant decrease in WBC levels in the blood of rats that were treated with TNBS, however, pure mesalamine showed no significant effect on other blood parameters. PM-DRGs significantly attenuated WBC count and upregulated RBCs and HGB levels only in the TNBS-induced UC rat model. PCM-DRGs significantly ameliorated WBC, RBC, HGB, and HCT% in the blood of TNBS-treated rats, however, this formulation showed no significant effect on the PLT count. Exceptionally, CM-DRG formulation significantly ameliorated all the blood parameters including WBC, RBC, HGB, HCT%, and PLT count in the TNBS-induced UC rat model.

Biomarkers of oxidative stress and inflammation were determined in the colon tissues of rats after 14 days of dosing. TNBS caused a significant increase in lipid peroxidation, and inflammation, and decreased the

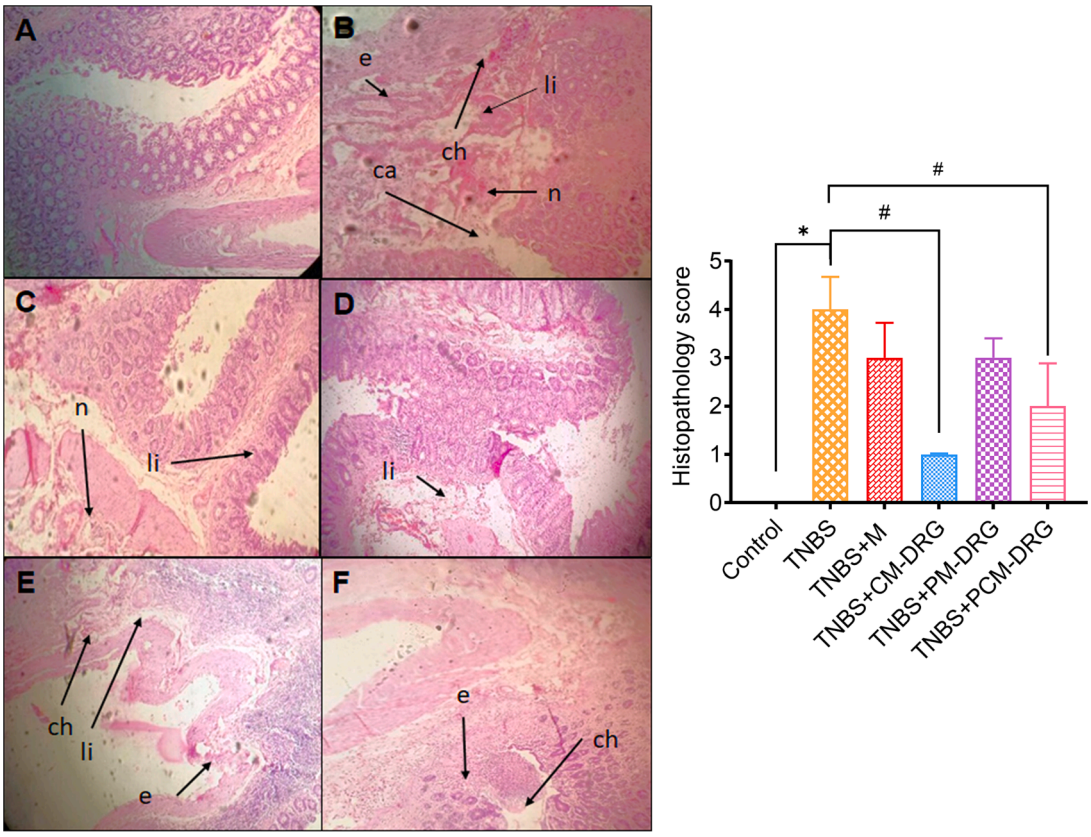

Fig. 11. Histopathology (H&E stain) of the colon of rat groups: (A) control, (B) TNBS, (C) TNBS + M, (D) TNBS + CM-DRG, (E) TNBS + PM-DRG, and (F) TNBS + PCM-DRG (×400, 100 μm). Values as expressed as Mean ± S.E.M. ($n = 5$ animal/group). A one-way ANOVA and Dunnett's multi-comparison test were used. * $p < 0.05$ *vs.* control and # $p < 0.05$ *vs.* TNBS group. n = necrosis, li = leukocyte infiltration, e = edema, ch = hyperplasia, M: Mesalamine, CM-DRG: Chitosan-mesalamine delayed-release granules, PM-DRG: Pectin-mesalamine delayed-release granules, PCM-DRG: Pectin-chitosan-mesalamine delayed-release granules.

levels of endogenous antioxidant activity in the colon of rats reminiscent of clinical features of UC. Earlier, an imbalance between pro-oxidants and anti-oxidants has been reported in UC (Krzystek-Korpacka et al., 2020). Infiltration of the colon mucosal barrier, submucosa, and lamina propria by neutrophils, lymphocytes, and eosinophils causes the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) which leads to an increase in pro-oxidants (Wang et al., 2016). These free radicals react with macromolecules and increase the permeability of the intestinal mucosa thereby aggravating the ongoing inflammatory reactions and also causing activation of proteolytic enzymes. Endogenous anti-oxidants such as GSH and SOD are the members of the first line of defense against these reactive species (Bourgonje et al., 2020). Intestinal microbes also contribute to ROS and an excess of ROS which exceeds the scavenging limits of endogenous anti-oxidants leads to colon damage. MDA is a secondary lipid peroxidation product that readily reacts with cell proteins and DNA. The damage instigates several cell death mechanisms such as necrosis and apoptosis (Krzystek-Korpacka et al., 2020). In the present study, mesalamine and mesalamine DRGs attenuated TNBS-induced increase in MDA levels and also improved the endogenous anti-oxidants such as GSH and SOD in the colon of rats. These results are similar to previous findings indicating relief in the pathogenesis of UC by curbing the pro-oxidative mechanisms in the TNBS experimental model (Hambardikar and Mandlik, 2022). Furthermore, UC is widely regarded as an autoimmune disorder with idiopathic etiology. Chronic hyperactivity of immune cells such as lymphocytes (CD4 + T-helper cells), neutrophils, eosinophils, and enzymes (*e.g.*, matrix metalloproteinases and myeloperoxidases) leads to an increase in pro-inflammatory cytokines, free radicals, and activation of inflammatory transcription factors such as nuclear factor kappa-B (NF-κB). The development and use of anti-inflammatory biologicals (*e.g.*, infliximab, Anakinra) which can antagonize the pro-inflammatory cytokines have been a cornerstone in the therapeutics of UC (Liso et al., 2022). High levels of IL-1β have been detected in clinical studies (Carty et al., 2000). Macrophages, dendritic cells, and monocytes generate IL-1β which increases intestinal epithelial tight junction permeability (Al-Sadi et al., 2008). IL-1β proliferates inflammation by inducing TNF-α and IL-6 and bridging the innate and adaptive immunity by instigation of Th1 and Th17 cells (De Mooij et al., 2017). Myeloperoxidase (MPO) is an enzyme found in neutrophils and is a biomarker of neutrophil infiltration. MPO reacts to form highly toxic products such as hypochlorous acid and tyrosyl radicals which deteriorate the normal tissues leading to oxidative stress and inflammation (Hansberry et al., 2017). Several studies have reported penetration of intestinal mucosa, submucosa, and lamina propria by neutrophils that lead to increased MPO activity and subsequent inflammation (Ahmad et al., 2020). Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that regulates antioxidants and inflammatory mechanisms. Under stress conditions, Nrf2 translocates to the nucleus and interacts with DNA to upregulate the transcription of antioxidant genes (Peng et al., 2023). The cooperative communication between Nrf2 and NF-κB helps in maintaining cellular homeostasis, attenuating immune response, and proteolytic enzyme activity (*e.g.*, metalloproteinases) (Liu et al., 2021). It maintains the integrity of intestinal membranes by facilitating the protective factors and also inhibiting intestinal fibrosis by regulating transforming growth factor-β1 (Peng et al., 2023; Liu et al., 2021). Overall, restraining the expression and activity of IL-1β and MPO, and upregulation of Nrf2 attenuate the pro-oxidative and inflammatory drive in the colon (Oliveira et al., 2021). Parallel to these findings, in this study, mesalamine and chitosan- and pectin-based mesalamine DRGs downregulated MPO activity and IL-1β levels and upregulated Nrf2 in

the colon of rats that were treated with TNBS. However, chitosan-mesalamine DRGs showed more pronounced effects on MPO, IL-1β, and Nrf2 activities with respect to all other treatments in the TNBS-induced experimental UC rat model.

5. Conclusions

In the current study, chitosan-mesalamine delayed-release granules (CM-DRGs) showed good compatibility, better flow and packing properties, and drug release patterns. Overall, CM-DRGs exhibited better efficacy in delivering the drug at the desired site in the colon with respect to the other formulations. Furthermore, *in-vivo* data indicated a significant amelioration of biomarkers of colon damage (*e.g.*, ulcer area, diarrhea), hematology parameters, oxidative stress, and inflammation in the colon of the TNBS-induced experimental UC rat model by CM-DRGs. The *in-vivo* ameliorative effects of CM-DRGs *via* anti-oxidant and anti-inflammatory activities were found to be more pronounced in experimental UC in comparison to other formulations. It can be inferred that chitosan-based delayed-release granules of mesalamine might offer better colon delivery of mesalamine *via* the oral route and have significantly improved therapeutic advantages against clinical UC.

CRediT authorship contribution statement

Imtiyaz Ahmed Najar: Investigation, Data curation, Writing – original draft, Writing – review & editing. **Archana Sharma:** Validation, Investigation. **Abdulrahman Alshammari:** Methodology, Funding acquisition. **Thamer H. Albekairi:** Data curation, Funding acquisition. **Metab Alharbi:** Writing – review & editing, Funding acquisition. **Taief Ahmad Dar:** Validation, Formal analysis. **Zulfkar Latief Qadrie:** Validation, Formal analysis. **Atul Kabra:** Data curation, Writing – review & editing, Visualization. **A.M.J Newton:** Conceptualization, Methodology, Validation, Resources, Visualization, Supervision, Project administration. **Manish Kumar:** Conceptualization, Methodology, Investigation, Data curation, Writing – review & editing, Visualization, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethics Statement

The animal protocol was approved by Institution Animals Ethics Committee (IAEC) (No. SSP/IAEC/17/03) of Swift School of Pharmacy, Punjab, India. All the experiments and animal care were in accordance with the regulations set forth by the Committee for the Control and Supervision of Experiments on Animals (CCSEA), the Government of India, and ARRIVE guidelines were followed.

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