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Synthesis and Characterization of Bacterially Triggered System of Aceclofenac for Colon Targeting

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KEYWORDS

Prodrug, ulcerative colitis, aceclofenac, inflammation, colon targeting, amino acids.

ABSTRACT:

Aceclofenac has been considered as the potential treatment against ulcerative colitis. When administered orally aceclofenac are commonly absorbed by the upper gut. The potency of this effective chemical is enhanced by forming the specific drug delivery to the affected area. As all ulcerative colitis and crohn's disease occurs in the large bowel (or colon), which is beyond the site of absorption, it is necessary to modify aceclofenac to prevent it being absorbed in the upper gut. Many oral formulations are designed to maximize aceclofenac release in the colon where it acts topically on the colonic mucosa. So, the objective of this project was to produce a safe, targeted and effective azo prodrugs of aceclofenac with amino acids to treat the ulcerative colitis. The ordinary treatment of inflammatory bowel disease requires the frequent intake of anti-inflammatory drugs at higher doses, which causes the absorption of these drugs from small intestine, leading to significant adverse effects. Therefore, out of the need to overcome this formidable barrier of GIT, colonic drug delivery has evolved as the ideal drug delivery system for the topical treatment of local diseases of colon like Crohn's disease or ulcerative colitis. To achieve successful colonic delivery, a drug needs to be protected from absorption and or the environment of upper GIT and then be abruptly released into proximal colon, which is considered as the optimum site for colon-targeted delivery of drug.

Introduction:

Certainly, the root cause behind the UC is still unclear but number of recent evidences reveals that over stimulation or insufficient management of the mucosal immune system act as a major pathophysiologic pathway. Consequently meticulous prominence has been given to either the study of mucosal inflammation or immunologic reactions. Lamina propria of the mucosa has becomes heavily infiltrated with a mixture of acute and chronic inflammatory cells in diseases state . Certainly there is prevalent raise in mucosal Immunoglobulant G (IgG) production, evidence of complement activation and activation of macrophages and T cells. This immunological activity is related with the release of a several group of cytokines, kinins, leukotriens, platelet activating factor (PAF) and reactive oxygenmetabolites. The defined mediators are not participate in the increasing of immune and inflammatory response, even thay also direct link with endothelial function, on endothelial function (which may increase permeability and lead to ischaemia) and on repair mechanisms, thus increasing collagen synthesis. Moreover, various other inflammatory mediators including cytokines (interleukins 1 and 6, tumor necrosis factor) will activate an acute phase response which leads to fever and rise in serum acute phase proteins. In-addition, some of the clinical characteristics of acute UC canbe explained by these mechanisms. It follows; therefore, any treatment which is capable to block the activation of these immunological and inflammatory effector mechanisms is significantly leads to a perfection in the patient's indications and to are duced in the inflammatory activity [10]. Prodrome symptoms included bloating, diarrhea, stomach pain, fever, weight loss and fatigue. Conversely, the time lap of prodromal period is very short in ulcerative colitis.[1,2]

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Approaches To Colon Targeted Drug Delivery Systems:

Prodrug Concept: There is a great emphasis on research to discover methods aimed at enhancing the efficacy of drugs and reducing their toxicity and unwanted side effects. A drug molecule with optimal structural configuration and physico-chemical properties for eliciting the desired therapeutic response may not necessarily possess the best molecular framework and properties for its delivery at the target site. Usually a small fraction of administered drug reaches the target area and since, most drugs also interact with non-target sites, an inefficient delivery may result in undesirable side effects [3]

Prodrug approach is widely applicable in various fields: Enhancing bioavailability and biomembrane passage. ii)prolongation of drug action., improving site specific drug delivery, improving organoleptic properties of a drug, decreasing side effects and toxicity of a drug,imparting depot activity to the drugs , reducing gastric irritability of a drug and improving drug stability *in vivo* and *in vitro*.[4]

Criteria for Selection of Drug for Colon Drug Delivery System (CDDS): The best Candidates for CDDS are drugs which show poor absorption from thestomach or intestine including peptides. The drugs used in the treatment of inflammatory bowel disease, ulcerative colitis, diarrhea, and colon cancer are ideal candidates for local colon delivery.[5] Oral administration of conventional dosage forms normally dissolves in the stomach fluid or intestinal fluid. It is a serious drawback in conditions where localized delivery of the drugs in the colon is required. Oral delivery of drugs to the colon is valuable in the treatment of diseases of colon. To overcome this limitation some drug carriers are used. Drug Carrier is another factor which influences CDDS. The selection of carrier for particular drugs depends on the physiochemical nature of the drug as well as the disease for which the system is to be used. Factors such as chemical nature, stability and partition coefficient of the drug and type of absorption enhancer chosen influence the carrier selection. Moreover, the choice of drug carrier depends on the functional groups of the drug molecule.For example, aniline or nitro groups on a drug may be used to link it to another benzene group through an azo bond. The carriers, which contain additives like polymers (may be used as matrices and hydro gels or coating agents) may influence the release properties and efficacy of the systems.[6,7]

Materials: Drugs aceclofenac, and mefenamic acid were obtained as gift sample from Alkem Laboratories, Mumbai, India. All other chemicals used in the synthesis were of A.R. grade and those of synthetic grade were purified prior to use.

Synthesis of azo conjugates of NSAIDs with amino acids:

1. Esterification of amino acids.
2. Synthesis of diazonium salt of amino acids.
3. Coupling of diazotised salt of amino acids with NSAIDs.

Synthesis of methyl ester hydrochlorides of amino acids: Freshly distilled (0.05 M, 6 ml) of thionyl chloride was slowly added to methanol (100 ml) with cooling and amino acid (0.1 M) was added to it. The mixture was refluxed for 6-8 h at 60-70ºC with continuous stirring on magnetic stirrer. Excess thionyl chloride and solvent was removed under reduced pressure giving crude amino acid methyl ester hydrochloride. It was treated with 20 ml portion of cold ether at 0ºC until the excess of dimethyl sulphate was removed. The resulting solid product was collected and dried under vacuum.[8,9]

Diazotization of amino acid: Amino acid ester (0.01 mol) was dissolved in a suitable volume of water containing 2.5-3 equivalents of hydrochloric acid (0.02 mol; 1.7 mL of 35%HCl), by the application of heat (if necessary) and then solution was cooled in ice. The temperature was maintained at 0-5^{0}C on a cryostatic bath and an aqueous solution of sodium nitrite (2 mol, 1.4 g in 10 mL) was added (portion wise), through syringe with complete assurance that the tip of the syringe was always dipped completely in the solution. The addition of sodium nitrite solution was continued till the solution gave an immediate positive test for excess of nitrous acid with an external indicator i.e. moist potassium iodide-starch paper.[10,11]

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Coupling of diazotised salt of amino acid with NSAIDs: Mefenamic acid (0.01 mol) was completely dissolved in sodium hydroxide solution (2 mol; 0.08g/mL). The solution was then cooled at below 5^0C. Then slowly diazotised salt of Amino acid was added with continuous stirring, through syringe. Alkaline condition was constantly maintained. After completing the reaction, water was evaporated and crude product was recovered. It was recrystallized by dissolving in methanol and cooling at 0^0C. Purified product was dried under vacuum. The reaction was monitored by TLC.[12,13]

In vitro hydrolysis study: In vitro hydrolysis studies of synthesized prodrugs were carried out in SGF at pH 1.2 (USP 1970). A solution of 10 mg of prodrug was prepared in 90 ml of SGF (pH 1.2). An aliquot of 15 ml of this solution was withdrawn repeatedly and kept in test tubes maintained at 37 ± 0.5ºC. At a definite interval of time (0.5, 1, 2 up to 6 h), an aliquot was withdrawn from different test tubes and was transferred to micro centrifuge tubes followed by addition of methanol to make up the volume. The tubes were placed in freezing mixture in order to arrest further hydrolysis, followed by vortexing at high speed for 5 min. After vortexing, the tubes were centrifuged at high speed (3000 rpm) for 5 min. A 5 ml of clear supernatant obtained from each tube was measured on UV spectrophotometer for the amount of free drug released after the hydrolysis of prodrugs in SGF. 6.8 g of monobasic potassium phosphate was dissolved in 250 ml of water, followed by the addition of 190 ml of 0.2 N sodium hydroxide and 400 ml of water. A 10 g of pancreatin was added and mixed. Water was added to make up the volume to 1000 ml. Procedure is same as the procedure explained in section[15,16]

In Rat Fecal Matter (pH 7.4): Male albino rats weighing 200-250 g and maintained on a normal diet were used for the study. Six rats were asphyxiated using carbon dioxide. The prodrug was dissolved in phosphate buffer (pH 7.4) so that final concentration of solution was 250 µg/ml. Fresh fecal material of rats were weighed (about 1 g) and placed in different sets of test tubes. To each test tube, 1 ml of the prodrug solution was added and diluted to 5 ml with phosphate buffer (50 µg/ml). The sets of test tubes were incubated at 37 ± 0.5ºC for different interval of time (0.5, 1, 2 up to 6 h). For analysis, the free drug was extracted with 5 ml of methanol and estimated on UV spectrophotometer[17,18]

In vivo Evaluation:

Ulcerogenic Activity: The ulcerogenic activity was determined by the Rainsford'scold stress method, which is an acute study model and is used to determine ulcerogenic potency of a given drug at ten time's higher dose. Mefenamic acid and sulfasalazine were taken as standards. It was found that the presence of suspending agents like carboxy methylcellulose decreases the incidence of gastric ulcers. Hence, the test compounds and standards were administered orally, as fine particles suspended in CMC by continuous stirring. The volume of vehicle or suspensions was kept constant. Wistar rats of either sex weighing between 200-250 g were randomly distributed in control and experimental group of five animals each. Doses of prodrugs were calculated on equimolar basis of drug . They were then converted into ten times higher doses. Following oral administration of 5 mL of the aqueous drug suspensions (at 10 times the normal dose), the animals were stressed by exposure to cold (-15 0C for 1 h). The animals were placed in separate polypropylene cages to ensure equal cold exposure. After 2 h of drug administration, the animals were sacrificed. The stomach and duodenal part were opened along the greater curvature and thenumber of lesions was examined by means of a magnifying lens. All ulcers larger than 0.5 mm were counted. The ulcers were scored according to the method and the ulcer index was determined[19-22]

TNBS induced experimental colitis model: Rats were fasted for 24 h before experimentation. Rats were lightly anesthetized with ketamine and xylazine (20mg/kg and 5mg/kg, i.m.). A polyethylene catheter with 2 mm diameter was inserted through the rectum intothe colon to a distance of 8 cm. For ulcerative colitis induction, TNBS dose was 150 mg/kg of body weight of TNBS in ethanol, 50% solution) was infused into the colon of all rats (except the normal control group) through the catheter, held in place for 30 sec. The catheter was left in place for few seconds then gentlyremoved. For 3 days the rats were housed without treatment to maintain the development of a full inflammatory bowel disease

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modelwith full access of food and water *ad libitum*. The animals of standard and test groups received orally drug and prodrugs respectively, once daily for five continuous days. The normal control and colitis control groups received only 1% carboxy methylcellulose instead of free drug or prodrug[23-25]

Assessment of colonic damage by clinical activity score: The animals of all groups were examined for weight loss, stool consistency and rectal bleeding throughout the 11 days study. Colitis activity was quantified with a clinical activity score assessing these parameters as previously applied by Hartmann. The clinical activity score was determined by calculating the average of the above three parameters for each day, for each group and was ranging from 0 (healthy) to 4 (maximal activity of colitis).[26]

Anti-inflammatory activity by Carragenan induced rat paw edema model: Male or female wistar rats with a body weight between 200-250g were used. The animals were stared overnight. These animals were treated with control, standard and synthetic test prodrug orally. Thirty minutes later, the rats were challenged by a subcutaneous injection of 0.05ml of 1% solution of carrageenan into planner side of the left hind paw. The paw volume was measured by venire caliper scale immediately after injection i.e 0 hrs to 5 hrs. The percent inhibition of paw oedema was calculated.[27-30]

Myeloperoxidase (MPO) activity determination: Myeloperoxidase (MPO) activity was determined by method of Krawisz .MPO activity is inversely proportional to the ameliorating effect on disrupted colonic architecture. The intestinal tissue samples (approximately 50 -100 mg) were homogenised on ice using a polytron (13,500 rpm, 1 min) in a solution of 0.5% hexadecyltrimethyl ammonium bromide (HTAB) in 50 mM potassium phosphate buffer (pH 6.0, 1 ml per 50 mg tissue). The resulting homogenate was subjected to three rapid freezing (70 ◦C) and thawing (immersion in warm water, 37 ◦C) cycles. The samples were then centrifuged (4000 rpm, 15 min, 4 ◦C) to remove insoluble material. The MPO containing supernatant (0.1 ml) was assayed spectrophotometrically after addition of 2.88 ml phosphate buffer (50 mM, pH 6.0) containing 0.167 mg/ml o-dianisidine hydrochloride and 0.0005% hydrogen perox- ide. The kinetics of absorbance changes at 470 nm was measured. Sample enzyme activity was calculated with a standard curve of known MPO unit activity. One unit of MPO activity, defined as the quantity of enzyme able to convert 1 mmol of hydrogen peroxide to water in 1 min at room temperature, was expressed in mU/100 mg of tissue. Myeloperoxidase (MPO) activity, which is an important quantitative index for inflammation was determined in terms of mU/100 mg tissue.[31-32]

Results & Discussion:

Azo prodrug of mefenamic acid-methionine (MMP): The physico- chemical and spectral charecterization of **MMP** are discussed as follows: M.pt. 263˚C, Rf- 0.7, percentage yield- 73%, aq. Solubility- 1.67mg/mL, log P- 0.72, ∧ max in HCl buffer (pH 1.2):287nm and in phosphate buffer (pH 7.4): 296nm.

(**MMP):** IR(KBr,cm^{-1}):2760(OH),3316 (NH), 3033 (C-H Ar),2931(CH_3),1678(C=O), 1638,1570,1486 and 1440 (C=C Ar),622(C-S), 1279(-OCH_2) 1490(N=N). ^{1}H NMR (DMSO-*d*6, 400 MHz, δ) Single spot TLC data of synthesized compound along with NMR values assured the purity of synthesized prodrug. 7.79-7.20 (4H, ArH), 8.80 (1H, NH), 10.2 (1H, COOH), 2.2 (1H, CH), 3.19 (4H, CH2), 3.67 (3H, OCH3), 1.35 (6H, CH3).

Azo prodrug of mefenamic acid-threonine (MTP): The physico- chemical and spectral charecterization of MTP are discussed as follows: M.pt- 259^{0}C, Rf- 0.6, percentage yield- 79%, Aq. Solubility- 1.43g/mL, logP- 0.76, max in HCl buffer (pH 1.2): 292 nm and in phosphate buffer (pH 7.4): 301 nm.

(**MTP):** IR (KBr, cm^{-1}): 2795 (OH), 3352 (NH), 3093 (C-H Ar), 2922 & 2876 (CH_3), 1689 (C=O), 1638,1570,1486 and 1440 (C=C Ar),1521 (N=N) 1289(- OCH_2)); ^{1}H NMR (DMSO-*d*6, 400 MHz, δ). Single spot TLC data of synthesized compound along with NMR values assured the purity of synthesized prodrug.NMR values: 7.29-7.56 (4H, ArH), 8.30 (1H, NH), 11.2 (1H, COOH), 2.4 (1H, CH), 3.11 (4H, CH2), 3.77 (3H, OCH3), 1.35 (6H, CH3).

Azo prodrug of mefenamic acid-leucine (MLP): The physico- chemical and spectral charecterization of MLP are discussed as follows: M.pt. 261˚C (decomposed), Rf- 0.75, percentage yield- 73%, aq. Solubility- 0.88 g/mL, log P- 1.21 , max in HCl buffer (pH 1.2) was 287 and in phosphate buffer (pH 7.4) was 341 nm.

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(**MLP):** IR (KBr, cm^{-1}): 2782 (OH), 3452 (NH),3022 (C-H Ar), 2921 & 2867 (CH_3), 1710 (C=O), 1631,1560,1496 and1450 (C=C Ar),1511 (N=N) 1259(- OCH_2)); ^1H NMR (DMSO-*d*6, 400 MHz, δ). Single spot TLC data of synthesized compound alongwith NMR values assured the purity of synthesized prodrug.7.39-7.66 (4H,ArH), 8.10 (1H, NH), 11.3 (1H, COOH), 2.3 (1H, CH) ,3.21 (4H, CH2), 3.67 (3H, OCH3), 1.29 (6H, CH3).

Azo prodrug of mefenamic acid-serine (MSP): The physico- chemical and spectral charecterization of MSP are discussed as follows: M.pt. 255˚C (decomposed), Rf- 0.65, percentage yield- 63%, aq. Solubility- 0.83 g/mL, log P- 1.21, max in HCl buffer (pH 1.2) was 277 in phosphate buffer (pH 7.4) was 293 nm.

(**MSP):** IR (KBr, cm^{-1}): 2792 (OH), 3432 (NH), 3012 (C-H Ar), 2941 & 2887 (CH_3), 1702 (C=O), 1601,1530,1476 and 1470 (C=C Ar),1501 (N=N) 1269(-OCH_2)); ^1H NMR (DMSO-*d*6, 400 MHz, δ). Single spot TLC data of synthesized compound alongwith NMR values assured the purity of synthesized prodrug. 7.89-7.71 (4H, ArH), 8.60 (1H, NH), 11.4 (1H, COOH), 2.5 (1H, CH), 3.13 (4H, CH2), 3.82 (3H, OCH3), 1.19 (6H, CH3)

In vitro kinetic evaluation: The negligible or none reversion was observed at gastric pH (SGF, pH 1.2) suggesting the stability of synthesized prodrugs in gastric pH, both in fasted and fed state. However at higher pH values i.e. in SIF representing intestine, the percentage reversion was significantly lower, thereby making the free drug available for absorption in the intestine which is 10.33% upto six hours. A much higher value was observed in rat fecal matter due to the enzyme dependant hydrolysis taking place in colon. Also, the process of reversion increases almost linearly with time at colonic pH.

TNBS induced experimental colitis model: The ulcerogenic activity of different NSAIDs associated prodrugs, was determined by TNBS induced experimental colitis model in rats. TNBS wasinfused into the colon of all rats (except the normal control group) through the catheter, held in place for 30 sec for induction of experimental colon ulcer. A significant appearance of experimental ulcer in animal colons which received TNBS treatment has been observed. In-addition, loss of body weight, stool consistency, rectal bleeding has observed on the basis of score. Additionally, the clinical activity score has been determined by calculating the average of above three parameters for every day against each group.

Summary &conclusion: Mefenamic acid has been considered as the potential treatment against ulcerative colitis. When administered orally Mefenamic acid is commonly absorbed by the upper gut. The potency of this effective chemical is enhanced by forming the specific drug delivery to the affected area. As all ulcerative colitis and crohn's disease occurs in the large bowel (or colon), which is beyond the site of absorption, it is necessary to modify Mefenamic acid to prevent it being absorbed in the upper gut. Many oral formulations are designed to maximize Mefenamic acid release in the colon where it acts topically on the colonic mucosa. So, the objective of this project was to produce a safe, targeted and effective azo prodrugs of Mefenamic acid with amino acids to treat the ulcerative colitis. Prodrug approach is one of the important approaches for targeting drugs to colon. Colon specific drug delivery through colon specific prodrug activation may be accomplished by the utilization of high activity of certain enzymes at the target site relative to non-target tissues for prodrug to drug conversion. The need for a totally safe, colon specific prodrug of NSAIDs with nontoxic carriers still remains. Therefore, it was thought worthwhile to design and synthesize new mutual azo prodrugs of NSAIDs with safe carrier amino acids. Overall results showed the better acceptability of MMP prodrug for treatment of ulcerative colitis.

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Table1: Percentage release of mefenamic acid in SIF

Time (min)	Prodrug Hydrolyzed in SIF(%)			
	MSP	**MLP**	**MTP**	**MMP**
15	0	0	0	0
30	1.11	1.12	1.14	1.03
45	2.14	2.3	2.5	2.42
60	3.17	3.19	3.23	3.41
75	4.23	4.29	4.39	4.22
90	5.37	5.79	5.53	5.81
105	7.03	7.52	7.43	7.65
120	7.81	7.94	7.98	7.91
240	9.53	9.44	9.97	9.94
360	10.16	10.12	10.15	10.33

Table 2: Percentage of drug released in rat fecal matter

Time (min)	% drug released			
	MSP	**MLP**	**MTP**	**MMP**
15	0	0	0	0
30	13.12	11.2	14.2	16.8
45	24.2	22.2	25.5	27.6
60	33.1	35.1	39.7	40.3
75	38.8	37.8	46.2	48.8
90	44.3	42.3	53.3	58.2

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105	53.3	53.3	68.2	69.2
120	63.8	61.8	73.1	75.4
240	76.6	75.6	79.9	83.6
360	87.3	88.3	89.7	92.1

Biological Evaluation:

Ulcerogenic Tendency:

Table 3: Results of ulcerogenic activity:

Compound	**Ulcer index ± S.D.**
Normal Control	0.6 ± 0.12
Diseases Control	28.4 ± 1.6
Standard (Sulfasalazine)	5.4 ± 0.15
Mefenamic acid	45.6 ± 1.8
MSP	5.8 ± 0.13
MLP	5..2 ± 0.2
MTP	4.8 ±0.97
MMP	4.8 ± 0.17

Table 4: Clinical activity score rate

	Days										
Groups	1	2	3	4	5	6	7	8	9	10	11
Normal control	0±0.02	0±0.02	0 ±0.02	0 ±0.02	0±0.02	0±0.02	0±0.2	0±0.02	0±0.02	0.1±0.02	0.1±0.02
Diseases control	0±0.02	0±0.02	0±0.02	3.1±0.02	4.0±0.5	3.8±0.02	3.5±.05	3.0±0.05	3.0±0.03	2.6±0.02	2.5±0.05
Standard	0±0.02	0±0.02	0 ±0.02	4.0±0.02	4.0±0.05	3.5±0.02	2.6±0.05	2.6±0.02	1.8±0.02	1.3±0.02	0.8±0.02
Mefenamic acid	0±0.02	0±0.02	0 ±0.02	4.0±0.05	4.0±0.02	3.5±0.03	2.5±0.05	2.6±0.02	2.0±0.05	1.4±0.02	1.0±0.02
MSP	0±0.02	0 ±0.02	0 ±0.02	4.0±0.05	3.5±0.02	3.5±0.05	3.1±0.05	2.5±0.05	1.2±0.05	0.5±0.04	0.5±0.05
MLP	0±0.01	0 ±0.05	0 ±0.02	3.6±0.02	3.5±0.04	3.1±0.05	2.5±0.04	2.0±0.05	1.5±0.02	0.8±0.05	0.4±0.03
MTP	0±0.0	0	0	3.5±0.0	3.4±0.0	3.2±0.0					0.5±0.0

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	2	±0.02	±0.02	2	2	5	2.5±0.05	2.0±0.01	1.6±0.02	1.2±0.02	4
MMP	0±0.02	0 ±0.02	0 ±0.02	4.0±0.04	3.5±0.02	3.1±0.05	2.6±0.02	2.0±0.05	1.5±0.03	0.8±0.02	0.4±0.02

Table 5: Colon to Body Weight Ratio

S. No.	Compound	Colon to body weight ratio (^{w}/w) ± S.D.
1	Normal control	0.006 ± 0.0004
2	Diseases control	0.04 ± 0.0005
3	Standard (Sulfasalazine)	0.009 ± 0.0006
4	Mefenamic acid	0.011 ± 0.0002
6	MSP	0.010 ± 0.0004
7	MLP	0.012 ± 0.0006
8	MTP	0.009 ± 0.0001
9	MMP	0.009 ± 0.0006

Table 6: Anti-inflammatory activity of NSAIDs and its prodrugs

Group	Prodrug	Anti-Inflammatory activity (%)				
		0.5 h	1 h	2 h	4 h	5 h
1	Normal Control	-	-	-	-	-
2	Standard (Sulfasalazine)	57.13 ± 1	64.19 ± 1.4	68.78 ± 1.7	73.2 ± 1.8	78.93 ± 2.4
3	Mefenamic acid	64.11 ± 2	63.12 ± 1	61.21 ± 2	50.40 ± 1	45.82 ± 1.1
5	MSP	51.15 ± 1.1	53.12 ± 2.1	57.31 ± 1.1	63.31 ± 2.1	68.12 ± 1.1
6	MLP	55.14 ± 1	61.42 ± 1.8	63.42 ± 1	67.71 ± 1.2	74.22 ± 2
7	MTP	56.12 ± 1	62.1 ± 1.2	65.7 ± 1.4	70.2 ± 1.4	77.33 ± 2.42
8	MMP	52.1 ± 1.4	58.4 ± 1.3	59.6 ± 1.5	68.2 ± 1	73.4 ± 2.35

Myeloperoxidase activity determination:

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Table 7: Myeloperoxidase activity in different TNBS induced colitis model

S. No.	Groups	MPO activity (mU/100mg tissue)
1	Healthy	20.3±2.18
2	Test (TNBS treated)	160.2±4.82
3	Standard(sulfasalazine)	53.6±4.29
4	Mefenamic acid	80.9±5.32
6	MLP	58.4±4.52
7	MSP	61.3±6.12
8	MTP	56.4±5.11
9	MMP	54.3±6.53