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Design, hydrolysis and pharmacological evaluation of novel polymeric prodrugs of Etodolac

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Abstract

Etodolac (ED), 1,8-diethyl-1,3,4,9-tetrahydropyrano-[3,4-b]indole-1-acetic acid, is a non-steroidal anti-inflamatory and antirheumatic drug. A biocompatible and biodegradable polymer dextran was used as a carrier in the study for synthesizing macromolecular etodolac prodrugs. The synthesis involves condensation of acyl imidazole derivatives of etodolac with dextran of different molecular weights (10000 and 20000) to obtain etodolac-dextran prodrugs ED10 and ED20 respectively. The structures were confirmed by IR and NMR spectroscopy and the degree of substitution was obtained as 13.3 % and 16 % for ED10 and ED20 respectively. Hydrolysis kinetics of the synthesized prodrugs were studied in borate buffer solutions (pH 7.4 and pH 9.0) and simulated colonic fluid (SCF, pH 6.8). Much faster hydrolysis was observed in SCF than borate buffer solutions and the hydrolysis followed first order kinetics. The pharmacological evaluation showed an enhanced anti-inflammatory activity of 61 % and 65 % inhibition of ED10 and ED20 against 52 % of parent drug. The analgesic activity of the prodrugs was insignificant, whereas a remarkable reduction in ulcerogenicity was observed. The improved aqueous solubility, increased therapeutic efficiency and reduced gastrointestinal side effects of the prodrugs confirm the use of dextran as a promoiety for the delivery of etodolac in colon.

Key words: Etodolac, macromolecular prodrug, anti-inflammatory, ulcer index

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Introduction

Etodolac (ED), 1, 8-diethyl-1,3,4,9-tetrahydropyrano-[3,4-b]indole-1-acetic acid, is one among the many well known non steroidal anti-inflammatory drugs (NSAIDs), which possess potential analgesic and anti-inflammatory activities. But it suffers from gastro intestinal (GI) disturbance,

peptic ulceration and GI bleeding [1-4]. The GI side effects produced by NSAIDs are either due to direct contact effect or indirect effect of drug on the GI mucosa. The major causes linked with GI untoward effects of NSAIDs are their acidic nature, ion trapping effect and inhibition of cytoprotective prostaglandins [5-8]. Literature reveals that chemical coupling of a polymeric, biodegradable carrier with drugs to form a polymeric or macromolecular prodrug might be a useful approach to improve the physicochemical properties and site specificity . Also in most of the macromolecular or polymeric prodrug approaches, the drug is either linked by physical entrapment or chemical linkage to polymeric carriers. Dextrans can be used as promoiety due to their excellent physico-chemical properties and physiological acceptance. The prodrug with the polymer can temporarily mask acidic function of ED and decrease its GI toxicity [9-12]. The present work aims at synthesizing dextran conjugates of etodolac and to evaluate their potential use as a polymeric prodrug for colon specific drug delivery. Also, *in vivo* investigations in animals were performed to assess their pharmacological effects and gastrointestinal toxicity.

Materials and Methods

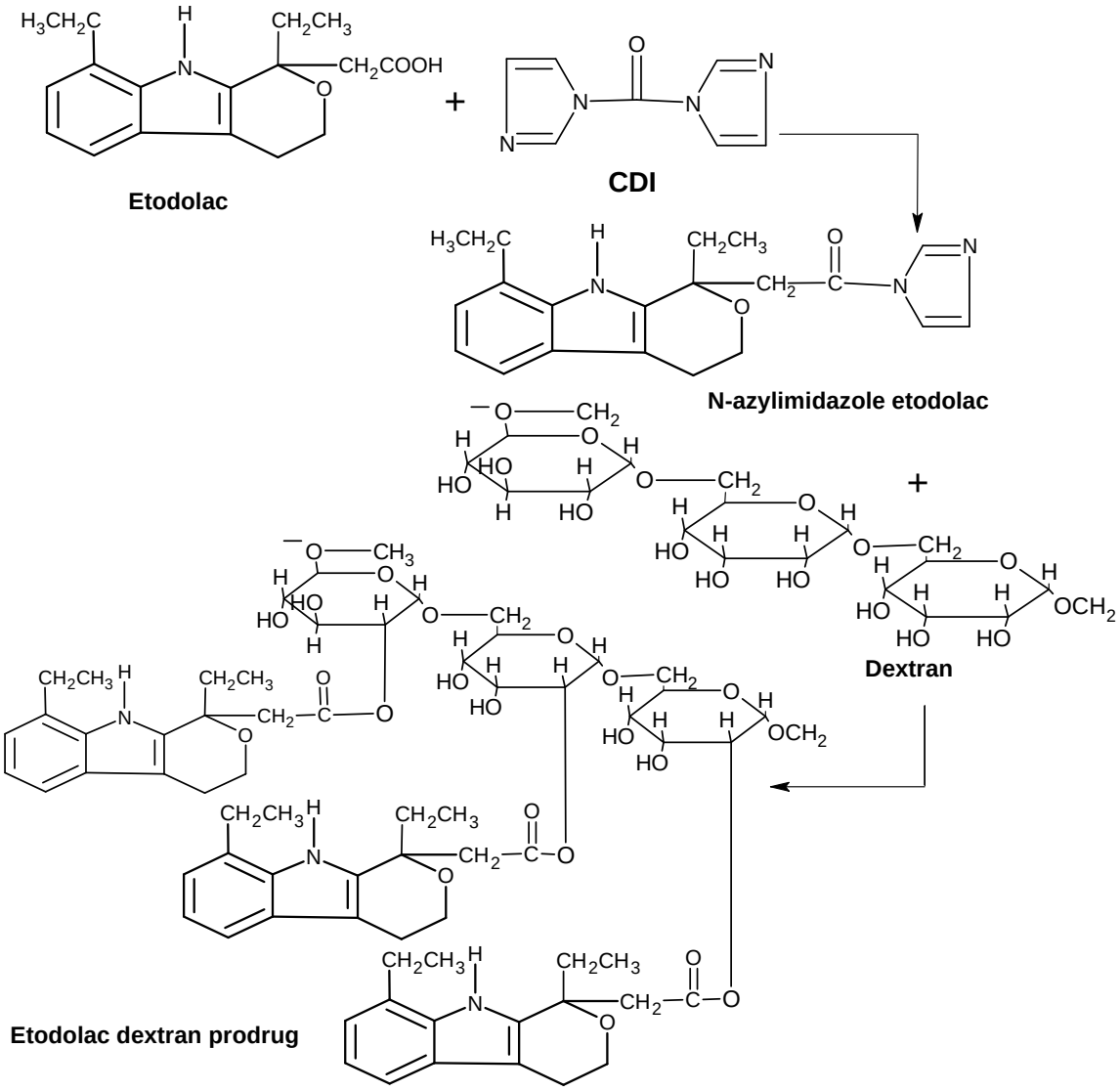
The etodolac was obtained as gift sample from Alkem Laboratories, India. Dextran (molecular weight - 10000 and 20000) and N,N–carbonyldiimidazole (CDI) were purchased from Sigma-Aldrich Chemicals Ltd, USA. Silica gel GF 254 for TLC was obtained from Sisco Laboratories, India. All other solvents and chemicals were of reagent grade and obtained from Qualinges fine chemicals, India. The melting points were recorded using melting point determination apparatus by Sigma Instrument, India. The IR spectra were recorded on Shimadzu 8300 FT-IR spectrophotometer (Japan) using KBr pellets in the range 4000 to 400 cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO on NMR spectrophotometer (Bruker DRX 300, USA). Chemical shifts are expressed as δ (ppm) values. The degree of substitution and hydrolysis studies were determined by Elico UV Spectrophotometer (India). The elemental analysis was performed using Carlo-Erba Model 1108 Analyzer (Italy) and the values found are almost near to that of the theoretical values.

Synthesis of Dextran Prodrugs

Dextran prodrugs of etodolac were prepared by first activating the carboxylic group using CDI to obtain etodolac acylimidazole (DDI), which were then condensed with dextran of different molecular weight (10000 and 20000) *in situ* to get ED10 and ED20 respectively (Scheme 1) [13]. The progress of the reaction was monitored by thin layer chromatography, which was performed on silica gel GF 254 as stationary phase and n-hexane: water: ethyl acetate: glacial acetic acid (30:10:10:2.5) as mobile phase. N,N–carbonyldiimidazole is moisture-sensitive and, therefore, dry solvents were used throughout and anhydrous conditions were maintained during the experiment.

Degree of Substitution

The degree of substitution of etodolac was determined [14] by dissolving 20 mg of the dextran prodrug in 20 ml solution of phosphate buffer (pH 9.0). The reaction mixture was maintained at 70 °C for 1h and left for 24 h for complete hydrolysis. It was then neutralized with 1N HCl. The amount of etodolac released during hydrolysis was extracted with chloroform and determined by UV spectrophotometer at the absorption maxima of 279 nm.



Scheme 1: Synthesis of etodolac-dextran prodrug

Molecular Weight

Intrinsic viscosities were estimated using Eq. 1. The average molecular weights were then calculated by Mark-Howink Sakurada equation (Eq 2).

$$[\eta] = [\eta \text{ rel-1}] / [c + 0.28\ c\ (\eta\text{rel-1})] \quad [1]$$

$$\log [\eta] = \log K + a \log M \quad [2]$$

where [η] represents intrinsic viscosity, η rel is the relative viscosity at concentration c (%, w/v), M is the molecular weight and K and a are the constants.

In-vitro Hydrolysis

In-vitro hydrolysis of the dextran prodrugs was studied in different borate buffer solutions (pH 7.4 and pH 9.0) and in SCF (pH 7.4). The rate of hydrolysis of the prodrugs was computed as the percentage drug hydrolyzed based on the cumulative amount of drug hydrolyzed divided by the total amount of drug contained in the prodrug. The rate of hydrolysis and half-life of the synthesized prodrugs were calculated using

$$r = (2.303/t) \log (b/b-x)$$

where r represents hydrolysis constant, t is the time in h, b is the initial concentration of prodrug, x is the amount of prodrug hydrolyzed and (b-x) is the amount of prodrug remaining.

Pharmacological Evaluations

ED as well as the synthesized prodrugs was evaluated for anti-inflammatory, analgesic, and ulcerogenic activities as well as histopathology. Test compounds and standard drugs were administered in the form of a suspension (1 % carboxymethylcellulose as a vehicle) by oral route of administration for anti-inflammatory and analgesic studies, but for ulcerogenicity intraperitoneally as suspension in 2 % (m/v) acacia. Wistar albino rats of four groups, including a control and a standard group, each with six animals were selected. The selected animals were housed in acrylic cages at standard environmental conditions at 25 ± 2 °C, relative humidity of 45–55 %, in a well ventilated room maintained at 12: 12 h light: dark cycle, fed with standard rodent diet and water *ad libitum*. All the animals were acclimatized for a week before experiment. All animal experiments were carried out according to the guidelines of the Committee for the Purpose of Control of Experiments on Animals and approval of the Institutional Animal Ethics Committee, Sree Vidyanikethan College of Pharmacy, Tirupati was obtained.

Anti-inflammatory Activity

The anti-inflammatory activity was evaluated using carrageenan-induced oedema of rat paw [15, 16]. Albino rats (100-200g) were divided into four groups of six animals each. Group 1 served as control group, group II received etodolac 2 mg/kg, group III and IV received prodrug ED10 and ED20 respectively, where the dose was molecularly equivalent to the free drug. The initial volume of right hind paw of albino rat was measured by plethysmometer without administration of drug. The drug was administered orally in 1% suspension of sodium CMC. After 30 min of drug administration of prodrug, carrageenen (0.1 ml, 1%) w/v solution in normal saline was injected into the planter surface of right hind paw of each animal as phlogistic agent. The volume of right hind paw of albino rats was measured after 1, 2 and 3 h. The mean difference in the volume of the right hind paw of rats was compared with control and standard. The percent inhibition of paw oedema was calculated as

$$\text{Percent inhibition} = (1-Vt/Vc) \times 100 \qquad [3]$$

where Vc – mean relative change in paw edema volume in control group and Vt - mean relative change in paw edema volume in test group.

Analgesic Activity

The analgesic activity of synthesized prodrugs was determined by thermal stimulus using tail flick method [17]. Analgesiometer was used for the determination of pain threshold of albino rats. The rat (100-200 g) was placed in a holder through which the tail of the rat was protruded out. The reaction time was recorded at 1, 2, 3 and 4 h after the treatment and cut-off time was 9 s. The normal reaction time, i.e. the time taken to flick the tail was noted. Animals showing delayed response were rejected. The prodrug was administered orally in 1% suspension of sodium CMC and compared with etodolac as reference. The percent analgesic activity was calculated by the formula given as

$$\% \text{ Analgesic activity} = [(T_2-T_1)/ (Tc-T_1)] \times 100 \quad [4]$$

where T_1 - the reaction time (s) before administration of prodrug and T_2 -the reaction time (s) after administration of prodrug and Tc - cutoff time in sec.

Ulcerogenicity

Gastrointestinal toxicity of the synthesized prodrugs was measured and compared with the parent drug by measuring ulcer index [18]. The prodrug was suspended in 10 ml of 2% w/v suspension of acacia. Measured volume of the suspension containing ED was administered orally to the test group daily for 5 days. The albino rats (100-200 g) were fasted after the administration of last dose, thereafter they were sacrificed by decapitation and the stomach was removed, opened and washed with distilled water. The lesions on the gastric mucosa were counted by visual examination using a binocular magnifier. Ulcers greater than 0.5 mm were recorded. The ulcer index (UI) was calculated by severity of gastric mucosal lesions which are graded as grade1 - less than1mm erosions, grade 2 - 1-2 mm erosions and grade 3 - more than 2 mm erosions. The UI was calculated as

UI = [1× (number of lesions of grade 1) + 2× (number of lesions of grade 2) + 3× (number of lesions of grade 3)]/10 [5]

Histopathological studies

The histopathological studies of stomach of rats [19] were carried out using haemotoxylin and eosin stain at Pathology Department, Sri Venkateswara Veterinary University, Tirupati, India. The stomach tissues were removed from the rats and fixed in 10 % normal saline for at least 48 h. These were then processed routinely and the tissues were embedded in paraffin wax. Histological sections were cut at 5-6 µm and stained with routine haematoxylin and eosin. These were then examined by a consultant histopathologist. The lesions observed were assessed for the following mucosal atrophy, the presence of inflammatory cells in the wall, oesinophils, lymphocytes and plasma cells. Photomicrographs of representative lesions at various magnifications were taken on Zeiss optical microscope (Germany), Stemi 2000-C, with a resolution of 10 x 45X, attached with trinocular camera.

Results and Discussion

The etodolac-dextran prodrugs ED10 and ED20 were synthesized (scheme 1) and the structures were confirmed by spectral methods. The physicochemical properties of prodrugs are given in Table 1.

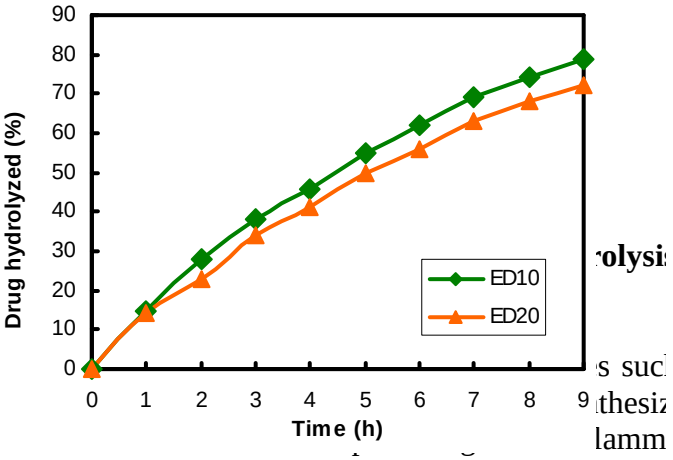
Table 1: Physicochemical properties of prodrugs

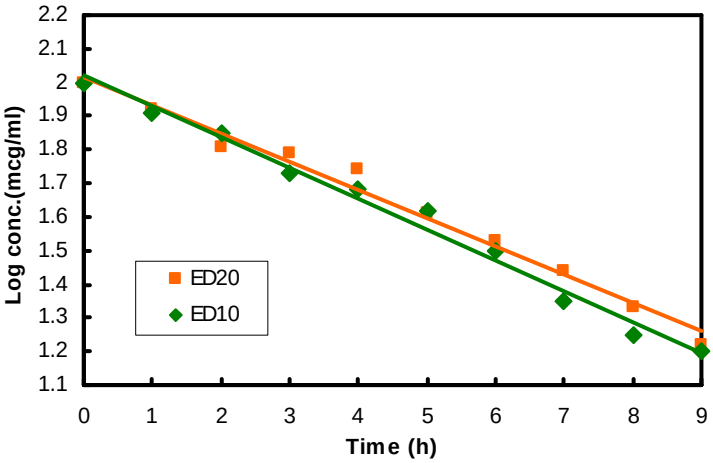
Prodrug	Colour	M.P. (^oC)*	Yield (%)	R_f # value	Degree of substitution[a]	Intrinsic viscosity	Molecular Weight	
							Calculated (%)	Found (%)
ED10	Creamy white	128-130	96	0.66	13.3	0.028	13861	15435
ED20	Creamy white	158-159	93	0.53	16	0.032	23450	24840

* Uncorrected # n-hexane: water: ethyl acetate: glacial acetic acid = 30:10:10:2.5
a - amount of parent drug in mg per 100 mg of prodrug

The IR and NMR spectral data of ED prodrugs are IR (KBr, max cm^{-1}): 1746 (C=O str.), 3057 (C-H str.), 736 (C-H aromatic bending), 3344 (-OH str.of polymeric-OH dextran), 1496 (str. of aromatic ring), 1278 (C-N str.). ^{1}H NMR (DMSO d6, ppm): 7.02 (m, 8H, aromatic ring), 3.2-3.74 (q, 2H, -CH2), 4.68-4.90 (m, anomeric protons of glucosidic ring). The IR spectrum of ED10 and ED20 shows characteristic stretching at 1746 cm^{-1} and confirms the formation of ester linkage. A strong OH stretching vibration of polymeric association at 3344 cm^{-1} and weak CH stretching of alkane at 3057 cm^{-1} were also found. It also showed the characteristic absorption stretch at 1496 and 1278 cm^{-1} for phenyl ring and C-N moieties respectively. The ^{1}H NMR analysis showed the characteristic shifting of signals of anomeric proton from δ 4.68 to δ 4.90 (1H), H-2 proton from δ 3.2 to δ 3.74, which indicates the formation of ester linkage at the position C-2. The signal of aromatic ring of etodolac was found at δ 7.02 and was in agreement with the anticipated structure. The disappearance of NMR signals in the range of δ 10.58 to δ 11.20 for carboxylic acid group in the etodolac-dextran prodrugs suggests that the free carboxylic group of drug was conjugated with hydroxyl group of dextran macromolecule and ester bond was formed.

Etodolac is poorly soluble in water, on the contrary, the prodrugs were found to be hydrophilic and soluble in water, acidic (pH 1.2) and basic media (pH 9). *In-vitro* chemical (aqueous medium pH 7.4 and 9) and enzymatic (SCF pH 6.8) hydrolysis kinetics of prodrugs were studied at 37 ± 1^0C. The studies indicated no hydrolysis at pH 1.2 for 3 h; hydrolysis of ED proceeds slowly at pH 7.4 and in SCF, whereas relatively much faster hydrolysis was observed. An absorption maximum in borate buffer (pH 9) was observed at 279 nm which was same as that of ED. The degree of substitution determined by UV spectrophotometry was found as 13.3 % for ED10 and 16% for ED20. The ED10 and ED20 showed negligible hydrolysis in acidic medium (pH 1.2) for 4 h. The hydrolysis of prodrugs at pH 7.4 demonstrated a slow rate of hydrolysis and relatively much faster hydrolysis was observed in SCF at pH 6.8 and also follows first order kinetics (Fig. 1). The half lives were found to be 3.73 h and 4.39 h for ED10 and ED20 respectively.





presented in Table 2. The synthesized prodrugs showed enhanced anti-inflammatory activity than the parent drug while the analgesic activity was not improved. An ulcer index of 17.66 for ED was observed which is on a higher range when compared with the ulcer index of 11 and 8.6 for ED10 and ED20 respectively (Fig. 2). The remarkable reduction in the ulcer index of the prodrugs indicated that dextran conjugation masked the gastrointestinal damaging tendency of etodolac.

Table 2: Pharmacological activities of ED10 and ED20

Prodrug	Anti-inflammatory activity		Analgesic activity		Mean Ulcer Index
	Paw volume (mL)[a]	Inhibition of oedema (%)	Basal reaction time (s)[a]	Pain reduction (%)	
Control	0.318 ± 0.550	-	26.17 ± 0.18	-	-
ED	0.145 ± 0.251	52	75.0 ± 0.75	75.0	17.6
ED10	0.057 ± 0.1	61	62.5 ± 0.50	62.5	11
ED20	0.058 ± 0.100	65	72.2 ± 0.20	72.2	8.6

[a]Each value represents the mean ± SD (n =6). Significance levels $p < 0.05$ as compared with the respective control.

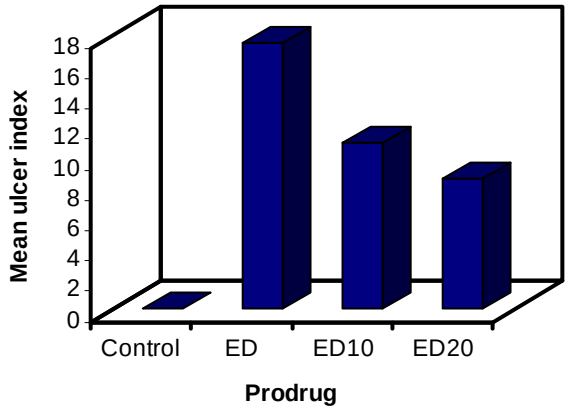


Fig. 2: Comparitive Ulcer Index of ED, ED10 and ED20

A normal histological finding was observed for the samples of the control group rats. Small hemorrhagic areas and patches of inflammatory cell infiltrations were present in the lumen of the glands and lamina propria when treated with parent drug, but normal histological findings were displayed for both ED10 and ED20 group. This reveals that the prodrugs are not producing any ulceration in the gastric region and are shown in Fig. 3.

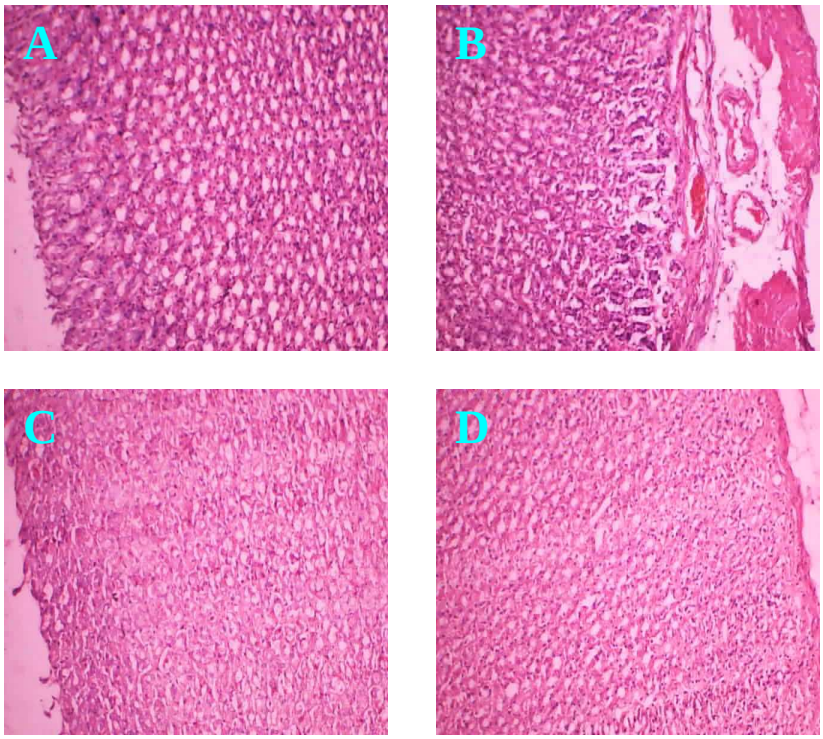


Fig. 3: Histopathological studies of prodrugs

A) Healthy control B) Ulcer control showing mucosal injury characterized by ED and massive mucosal infiltration of inflammatory cell C) Treated with ED10 D) Treated with ED20

Conclusion

The ED10 and ED20 were successfully synthesized and the structures were confirmed by spectral analysis. Both prodrugs showed encouraging hydrolysis rate in SCF and excellent pharmacological response. Increased anti-inflammatory as well as reduction in ulcer index of the prodrugs were observed when compared to the parent drug. The histopathological findings reveled that there is limited ulcer formation in stomach by the prodrugs. In conclusion, the present investigations suggest that dextran can successfully be employed as promoiety for compound containing a carboxylic function. The study also proposes dextran as a polymeric carrier to achieve colon site specificity due to the presence of enzymes and alkaline pH in the colon, improved physiochemical properties and reduced gastrointestinal side effects.

Statistical analysis

Statistical analysis of the pharmacological activity of the synthesized prodrugs on animals was evaluated using a one-way analysis of variance (ANOVA). Student's t-test was applied for

expressing the significance and the experimental data are expressed as mean ± SD (standard deviation).

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