

## DEXTRAN-ETODOLAC CONJUGATES: SYNTHESIS, *IN VITRO* AND *IN VIVO* EVALUATION

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**Abstract:** Etodolac (E), is a non-narcotic analgesic and antiinflammatory drug. A biodegradable polymer dextran has been utilized as a carrier for synthesis of etodolac-dextran conjugates (ED) to improve its aqueous solubility and reduce gastrointestinal side effects. An activated moiety, i.e. N-acylimidazole derivative of etodolac (EAI), was condensed with the polysaccharide polymer dextran of different molecular weights (40000, 60000, 110000 and 200000). IR spectral data confirmed formation of ester bonding in the conjugates. Etodolac contents were evaluated by UV-spectrophotometric analysis. The molecular weights were determined by measuring viscosity using the Mark-Howink-Sakurada equation. *In vitro* hydrolysis of ED was done in aqueous buffers (pH 1.2, 7.4, 9) and in 80% (v/v) human plasma (pH 7.4). At pH 9, a higher rate of etodolac release from ED was observed as compared to aqueous buffer of pH 7.4 and 80% human plasma (pH 7.4), following first-order kinetics. *In vivo* investigations were performed in animals. Acute analgesic and antiinflammatory activities were ascertained using acetic acid induced writhing model (mice) and carrageenan-induced rat paw edema model, respectively. In comparison to control, E and ED1-ED4 showed highly significant analgesic and antiinflammatory activities ( $p < 0.001$ ). Biological evaluation suggested that conjugates (ED1-ED4) retained comparable analgesic and antiinflammatory activities with remarkably reduced ulcerogenicity as compared to their parent drug – etodolac.

**Keywords:** dextran-etodolac conjugates, dextran carrier, NSAIDs

Etodolac is a racemic acetic acid derivative, non-selective cyclooxygenase (COX) inhibitor with potential analgesic and antiinflammatory activities. It is effective in the treatment of osteoarthritis, gout, rheumatoid arthritis and traumatic injury. Administration of non-selective COX inhibitor by oral route causes many gastrointestinal (GI) side effects like nausea, vomiting, dyspepsia, gastric irritation, peptic ulceration and bleeding (1). Owing to these common gastrointestinal side-effects, the NSAIDs frequently cause gastrointestinal injury and increase the risk of ulcer complications. There were some data suggesting that etodolac, like other NSAIDs, produces some gastrointestinal side effects (2-5). GI side effects produced by NSAIDs are either due to direct contact or indirect effect of the 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 (6-8). 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 physicochemical properties and clinical acceptance of drugs (9-12), parent drug is then released *in vivo* through biotransformation.

A polysaccharide macromolecule, dextran, has been used in clinical practice due to its better physiological acceptance and low toxicity. It has excellent physicochemical features such as high aqueous solubility, numerous hydroxyl groups for drug conjugation and availability in a wide range of molecular weights imparted to be used as a model carrier (13, 14). Literature revealed that many synthesized dextran conjugates showed improved physicochemical properties, targeted drug delivery and colon specificity (15-19). Our present work is an attempt to synthesize dextran conjugates of etodolac and evaluation of their potential use as a polymeric prodrug for oral drug delivery. Also, *in vivo* investigations in animals were performed to assess their pharmacological effects and gastrointestinal toxicity.

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## EXPERIMENTAL

Etodolac was a generous gift from Wyeth Ayerst (USA). Dextran of different molecular weights and *N,N'*-carbonyldiimidazole (CDI) were purchased from Fluka Biochemika (Switzerland). Purity of the compounds was tested by thin layer chromatography (TLC) on silica gel-G coated plates (Merck, India) using iodine vapor as visualizing agent. Carrageenan was purchased from Hi-Media Laboratories Pvt. Ltd. (India). Other chemicals were of synthetic grade. Hydrochloric acid buffer (pH 1.2), phosphate buffer (pH 7.4) and borate buffer (pH 9) were used for *in vitro* hydrolysis studies.

### Synthesis of dextran-etodolac conjugates

To a solution of etodolac (0.56 g,  $1.96 \times 10^{-3}$  mol) in dry DMSO (4 mL), CDI (0.45 g,  $2.78 \times 10^{-3}$  mol) was added slowly in portions for 30 min, maintaining the temperature of the reaction at 0°C. A solution of dextran (1 g in dry DMSO, 15 mL) of different molecular weights (40000, 60000, 110000 and 200000) was added to the above mixture with stirring, maintaining the reaction at 10°C for 30 min and then it continued for 3 days at room temperature in a dry area with occasional stirring. The conjugates were precipitated by addition of a methanol : diethyl ether (1:1, v/v) mixture successively for 4–5 times to remove unconjugated drug and then washing with acetone to obtain dextran-etodolac conjugates ED1, ED2, ED3 and ED4 (Scheme 1).

### Physicochemical analysis

Purity and absence of entrapped free drug in the conjugates was confirmed by TLC on silica gel-

G plates. Chloroform/methanol/glacial acetic acid (5: 0.5: 0.1, v/v/v) was used as a mobile phase and iodine vapors were used for spot detection. The UV spectra were recorded on a double beam UV-Vis spectrophotometer-160 (Shimadzu, Japan). The  $\lambda_{\max}$  value in borate buffer (pH 9) was found at 278.2 nm. The IR spectra of the samples were recorded on a Perkin-Elmer IR spectrophotometer (UK) in KBr discs. Elemental analyses (C, H, N) were carried out on a Carlo-Erba model 1108 analyzer (Italy). The physicochemical characterization data of ED1- ED4 are presented in Table 1. The average molecular weight was evaluated by intrinsic viscosity measurements of the conjugates (2%, w/v aqueous solution) using a capillary viscometer, at 27°C (single measurement at one concentration). Intrinsic viscosities were estimated using Eq. 1. The average molecular weights were then calculated by the Mark-Howink- Sakurada equation (20) (Eq. 2).

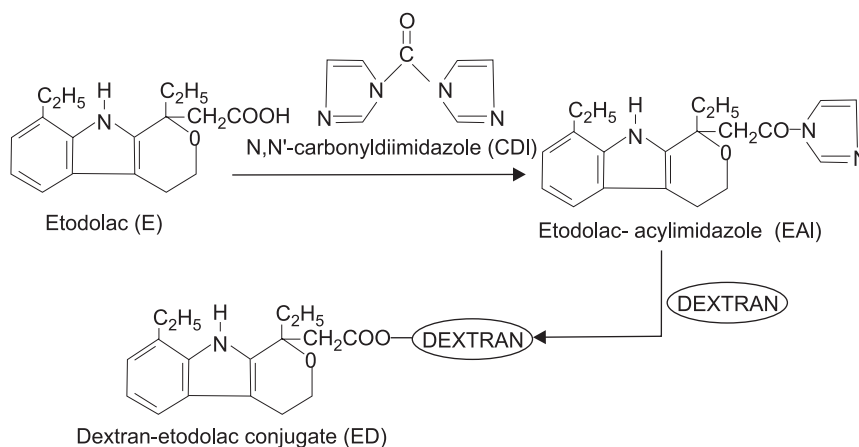
$$[\eta] = [\eta_{rel} - 1] / [c + 0.28 c (\eta_{rel} - 1)] \quad [1]$$

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

where  $[\eta]$  represents intrinsic viscosity,  $\eta_{rel}$  – relative viscosity at concentration  $c$  (% w/v),  $M$  is the molecular weight and  $K$  and  $a$  – are the constants.

### Drug content

The etodolac content (mg per 100 mg of dextran conjugate) was estimated. Dextran-etodolac conjugate (1 mg/mL) was dissolved in aqueous borate buffer (pH 9). The reaction was maintained at 70°C for 1 h. The solution was then left aside for 24 h at room temperature for complete hydrolysis. After neutralization with 1 mol/L HCl, the released etodolac was extracted in chloroform and estimated at the absorption maximum of 273.4 nm.



Scheme 1. Synthesis of dextran-etodolac conjugates.

### ***In vitro* hydrolysis profile**

Hydrolysis studies at pH 9. – Ten aliquots of 10 mg of ED were dissolved in 10 mL of borate buffer (pH 9) in test tubes. The reaction temperature was maintained at  $37 \pm 1^\circ\text{C}$  in a water bath. Test tubes were withdrawn at regular time intervals (10 min) till 1 h. The solution was cooled to room temperature and neutralized with 1 mol/L HCl. The regenerated etodolac upon hydrolysis was extracted in chloroform and determined at 273.4 nm.

Hydrolysis studies at pH 1.2, 7.4 and in 80% human plasma (pH 7.4). – Samples of ED (2 mg/mL) in aqueous medium (pH 1.2, pH 7.4 or 80% human plasma – pH 7.4) were maintained at  $37 \pm 1^\circ\text{C}$ . Aliquots were withdrawn after fixed intervals of 0.5, 1, 2, and 3 h and the released etodolac was estimated using the same method as described for hydrolysis at pH 9.

### **Pharmacological screening**

Animal handling and *in vivo* animal studies were carried out according to the Institutional Good Laboratory Practice, M. G. M. Medical College, India. Wistar rats (150–200 g) and Swiss albino mice (20–25 g) of either sex were used for biological screening. Animals were acclimatized to laboratory conditions for at least 3 days before commencement of the experiments and were kept under a 12 h light/12 h dark cycle. Animals were fasted overnight prior to treatment and received free access to water during the experiment. Doses of ED1-ED4 were equimolar to the parent drug (etodolac), which was calculated on the basis of their drug content in the conjugates. All the drugs were prepared in 2% gum acacia and administered orally (*po* route through oral gavage).

### **Analgesic activity**

Acute analgesia produced by the drugs was assessed by the acetic acid induced writhing method in mice (21). Mice were divided into three groups ( $n = 6$  in each group). Group I served as a control (received an appropriate volume of 2% gum acacia, *po*). The group II was a standard (etodolac 3 mg/kg body weight (b.w.), *po*) and group III, *i.e.* a test group, received ED (ED1-ED4, 75, 86, 60, or 75 mg/kg b.w., *po*, respectively). Three hours after treatment, 0.6% (v/v) acetic acid solution (10 mL/kg) was injected to mice intraperitoneally. Total number of writhes, which was a parameter of chemically induced pain (*i.e.* constriction of abdomen, turning of trunk and extension of hind limbs), was counted for 20 min. The analgesic effect was expressed as percent reduction of writhes in comparison with the control.

### **Antiinflammatory activity**

The carrageenan induced rat hind paw edema method described by Winter et al. (22) was used to evaluate the acute antiinflammatory activity of the conjugates. Rats were divided into control, standard and test groups of six animals each. Pretreatment initial paw volumes of all animals was measured using a mercury plethysmometer. The control group was given only an appropriate volume of 2% gum acacia orally. Standard group received etodolac (3 mg/kg b.w., *po*). To the test group, ED conjugates were administered orally using the similar doses as employed in the analgesic activity. One hour after treatment, edema in the left hind paw of the rat was induced by injection of 0.1 mL of 1% (w/v) carrageenan solution in distilled water. The relative change in paw volume was determined by measur-

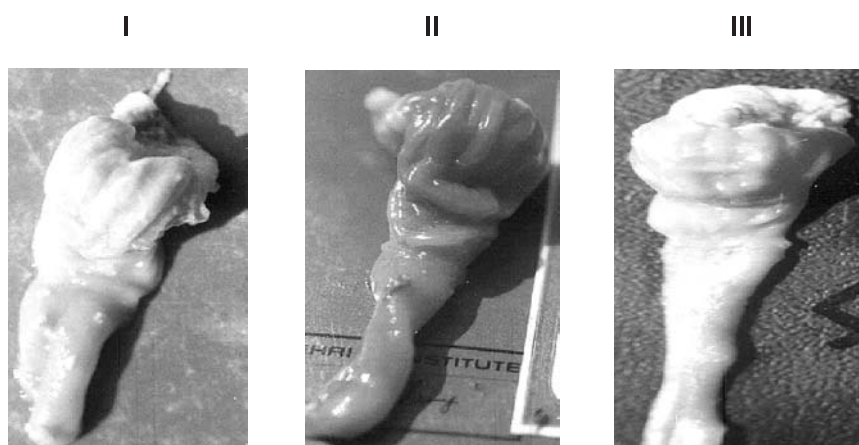


Figure 1. Ulcerogenic activity of etodolac and dextran-etodolac conjugates: I – control; II – etodolac; III – etodolac-dextran conjugate.

ing the paw volume after 3 h following the carageenan administration. The percent inhibition of edema, as an indication of antiinflammatory activity was compared with the controls.

#### Ulcerogenic activity

It was assessed using the method adopted from Shanbhag et al. (23). Rats were randomly distributed into three groups (n = 6 in each group). The control group received no drug while the standard group was given etodolac (60 mg kg<sup>-1</sup> b.w., *po*) and the test group received ED1-ED4 (1500, 1720, 1200, or 1500 mg/kg b.w. respectively, *po*) for 3 days. The animals were fasted 8 h pre- and 4 h post-treatment. Food and water were available for the rest of the time. Four hours after the last dose, the animals were sacrificed. The stomach with 3 cm of duodenum was removed, opened and washed with distilled water. The mucus was wiped off and the number of lesions was examined. The degree of mucosal damage and ulceration was scored as + (strong irritation identified by redness and inflammation), ++ (ulcers < 0.5 mm), +++ (ulcers > 0.5 mm) and ++++ (perforation) (Fig. 1).

#### Statistical data analysis

The data were expressed as the mean  $\pm$  S.D. and statistically evaluated by Student's t-test, *p* < 0.05 was considered as statistically significant.

## RESULTS AND DISCUSSION

Dextran-etodolac conjugate was obtained in two steps. Firstly, reaction of etodolac with CDI produced an activated N-acylimidazole of etodolac (EAI). In second step EAI condensed with dextran of different molecular weight (40000, 60000, 110000 or 200000) afforded the corresponding ED1, ED2, ED3 or ED4 conjugates (Scheme 1). Due to

the moisture sensitivity of CDI, the reaction was carried out under anhydrous conditions.

The IR spectrum revealed the presence of characteristic absorption bands at 3300 cm<sup>-1</sup> (OH broad) and 1730 cm<sup>-1</sup> (C=O ester) and confirmed the formation of ester linkage. The 'drug content' *i.e.* the amount of etodolac in the conjugates, estimated by UV-spectrophotometry after complete hydrolysis of ED in borate buffer (pH 9) was found to be between 3.5 and 5% (w/w). The average molecular weight was calculated by the Mark-Howink-Sakurada equation (20) using viscosity method (Table 1). It was observed that the variable molecular weights of dextran did not influence the drug content. Etodolac is poorly soluble in water, on the contrary, the ED was found to be hydrophilic and soluble in water, acidic (pH 1.2) and basic media (pH 9).

*In vitro* chemical (aqueous medium – pH 1.2, 7.4 and 9) and enzymatic (80% human plasma pH 7.4) hydrolysis kinetics of ED was studied at 37  $\pm$  1°C. The studies indicated no hydrolysis at pH 1.2 for 3 h; hydrolysis of ED proceeds slowly at pH 7.4 and in 80% human plasma, whereas relatively much faster hydrolysis was observed at pH 9. The corresponding half-lives for ED1, ED2, ED3 or ED4 were found to be 69.9, 52.1, 55.5, 80.9 h (in phosphate buffer pH 7.4); 59.7, 45.3, 47.1, 73.0 h (in 80% human plasma – pH 7.4) and 3.9, 3.6, 2.9, 4.5 h. (in borate buffer – pH 9), respectively. The results also indicated that hydrolysis did not affect the molecular weights of carrier dextran. The fact that hydrolysis kinetics showed nearly similar half-lives for ED at pH 7.4 and human plasma (80% v/v) suggested that hydrolysis is not catalyzed by plasma enzymes. High stability of dextran conjugates in acidic pH was also suggested by Ahmad et al. (24) and Shrivastava et al. (25). The high susceptibility of dextran conjugates to hydrolysis in highly alkaline medium may be attributed to intramolecular

Table 1. Physicochemical characterization data of dextran-etodolac conjugates.

Conjugate	Drug content (% w/w) <sup>a</sup>	Molecular weights		Elemental analysis (%)					
		Calculated <sup>b</sup>	Found	Calculated			Found		
				C	H	N	C	H	N
ED1	4	42421	45217	46.66	5.70	0.33	46.80	5.93	0.38
ED2	3.5	63178	64495	46.43	5.69	0.33	48.63	5.39	0.34
ED3	5	118488	120613	47.11	5.73	0.40	47.46	5.64	0.43
ED4	4	212099	213825	46.66	5.70	0.33	46.83	5.79	0.37

ED: dextran-etodolac conjugates. <sup>a</sup> Amount of etodolac in mg per 100 mg of dextran-etodolac conjugate. <sup>b</sup> Calculated on the basis of drug content.

Table 2. Analgesic and antiinflammatory activities of etodolac and dextran-etodolac conjugates.

Treatment	Dose <sup>a</sup> ( <i>po</i> , mg/kg b.w.)	Analgesic activity		Anti-inflammatory activity	
		Number of writhes <sup>b</sup>	Pain reduction (%)	Paw volume (mL) <sup>b,c</sup>	Inhibition of edema (%)
Control	—	55 ± 3.7	—	0.90 ± 0.05	—
Etodolac	3	32 ± 4.0 <sup>d</sup>	41.8	0.20 ± 0.12 <sup>d</sup>	77.8
ED1	75	37 ± 2.3 <sup>d,f</sup>	32.7	0.27 ± 0.10 <sup>d,g</sup>	70.0
ED2	86	35 ± 3.2 <sup>d,e</sup>	36.3	0.25 ± 0.16 <sup>d,g</sup>	72.2
ED3	60	34 ± 5.5 <sup>d,e</sup>	38.2	0.24 ± 0.15 <sup>d,g</sup>	73.3
ED4	75	38 ± 4.6 <sup>d,f</sup>	30.9	0.28 ± 0.20 <sup>d,g</sup>	68.9

ED: dextran-etodolac conjugate. <sup>a</sup> Dose equimolar to the parent drug (etodolac) calculated on the basis of drug contents. <sup>b</sup> The mean ± SD, n = 6, <sup>c</sup> Difference in paw volume 3 h after carrageenan injection. <sup>d</sup> p < 0.001 compared to control, <sup>e</sup> p > 0.05 compared to etodolac, <sup>f</sup> p < 0.05 compared to etodolac, <sup>g</sup> p > 0.05 compared to etodolac.

Table 3. Ulcerogenic activity of etodolac and dextran-etodolac conjugates.

Treatment	Dose <sup>a</sup> ( <i>po</i> , mg/kg b.w.)	Number of ulcers <sup>b</sup>	Degree of ulceration <sup>c</sup>
Control	—	0	0
Etodolac	60	7 ± 3.2	+, ++, +++, +++++
ED1	1500	0	+
ED2	1720	0	+
ED3	1200	0	+
ED4	1500	0	+

ED: dextran-etodolac conjugates. <sup>a</sup> Dose equimolar to the parent drug (etodolac) calculated on the basis of drug contents; <sup>b</sup> ulcers > 0.5 mm; the mean ± SD, n = 6; <sup>c</sup> +: strong irritation, ++: ulcers < 0.5 mm, +++: ulcers > 0.5 mm, +++++: perforation.

catalysis by neighboring hydroxyl groups or it might be related to the basic character of the carbohydrate alkoxide ions. A lack of plasma enzyme-mediated hydrolysis is most likely due to the steric hindrance by the dextran backbone (26).

Dextran conjugates ED were screened for analgesic, antiinflammatory and ulcerogenic activities in animals. The acetic acid-induced writhing model was used for assessment of analgesic activity in E and ED1-ED4. Highly significant reduction in number of writhes was observed after treatment with etodolac and ED1-ED4 in comparison to control group (p < 0.001). The results also showed that there were no significant variations in analgesic activity of ED2 and ED3 in comparison to etodolac (p > 0.05), whereas ED1 and ED4 showed slight variation (p < 0.05). However, the results indicated that at equimolar doses ED1-ED4 retained comparable analgesic activity with that of etodolac.

The standard drug etodolac showed 42% analgesic activity, whereas etodolac-dextran conjugates (at equimolar doses to etodolac, orally) showed analgesic activity ranging from 30 to 38 %.

The percentage inhibition of carrageenan-induced rat hind paw edema three hours post-dosing of etodolac was found to be 77% while etodolac-dextran conjugates (equimolar doses to etodolac) showed 69 to 73 % inhibition in ED1-ED4, respectively (Table 2). All the conjugates (ED1-ED4) and etodolac showed highly significant antiinflammatory activity in comparison to control group (p < 0.001). The evaluation also demonstrated that there was no statistically significant variation in ED1-ED4 in comparison to standard etodolac (p > 0.05). The results suggest that the conjugates retained comparable antiinflammatory activities as that of the parent drug. Animals did not show any gross behavioral changes, sedation, morbidity or mortality for 72 h at the administered oral doses of etodolac or its conjugates used for analgesic and antiinflammatory activities.

The investigations of ulcerogenic activity indicated that all the etodolac dextran conjugates showed a remarkable decrease in ulcerogenic property compared to their parent drug. Etodolac caused gastrointestinal damage and ulcers, in contrast,

ED1-ED4 showed only irritation without any ulceration (Table 3). The results indicated that dextran conjugation further masked the gastrointestinal damaging tendency of etodolac.

## CONCLUSION

In conclusion, the present investigations suggest that the dextran can be employed as carrier to obtain dextran-etodolac conjugates, which may represent potentially useful conjugates for oral administration of etodolac with improved aqueous solubility and remarkably diminished gastrointestinal side effects, while retaining comparable biological activities of the parent drug.

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