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Diclofenac-induced Gastric Ulceration in Rats: Protective Roles of Pantoprazole and Misoprostol

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EEZED and MMG designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors HAY and SG managed the literature searches, analyses of the study performed the spectroscopy analysis. Authors NAEM and MAER managed the experimental process. All authors read and approved the final manuscript.

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ABSTRACT

Gastrointestinal damage caused by diclofenac remains a significant clinical problem. Pantoprazole provides potent and long-lasting inhibition of gastric acid secretion and has proven efficacy in healing diclofenac-associated ulcers, including those with continued exposure to diclofenac. The objective of this study was to prepare and evaluate microbeads of diclofenac sodium coated with sodium alginate and Hydroxypropylmethylcellulose (HPMC) in order to obtain controlled release drug delivery system. The ulcerogenic activity and histopathological effects of the prepared formulation were compared with a marked formula containing the drug with misoprostol which orally administered to male Wistar rats. Ionotropic gelation technique was the technique of choice to encapsulate the drug. IR spectral analysis indicated no interaction between the drug and polymers used. Microbeads which showed a significant reduction in the release of diclofenac at acidic pH of

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the stomach as well as maximal release at alkaline pH of the intestine were selected to conduct further *in vivo* evaluation. The beads were administered to rats in combination with pantoprazole. The obtained ulcer index as well as the histopathological effects of the proposed formulations was compared to marketed formula containing the drug combined with misoprostol. The obtained *in vivo* results indicate that administration of pantoprazole and diclofenac microbeads has shown efficacy in reducing the risk of GIT ulcerations compared with administration of misoprostol and diclofenac or diclofenac separately.

Keywords: Diclofenac sodium; microbeads; ionotropic gelation technique; ulcerogenic effect of diclofenac; controlled release system; pantoprazole; misoprostol.

1. INTRODUCTION

Among different disorders of gastrointestinal system, peptic ulcer is the one which is more prevalent and have greatest clinical impact. Ulcer is characterized by disruption of mucosal integrity leading to local defect or excavation due to active inflammation [1]. Pathophysiology of ulcer is due to an imbalance between aggressive factors (acid, pepsin, H. pylori and NSAID's) and local mucosal defensive factors (mucus bicarbonate, blood flow and prostaglandins). Integrity of gastro duodenal mucosa is maintained through a homeostatic balance between these aggressive and defensive factors [2].

Clinically, regulation of gastric acid secretion is considered as major therapeutic target in the management of disease. Among clinically established drugs, H_2 blockers and proton pump inhibitors are most widely used as anti-ulcer drugs in addition to the cytoprotective agents like sucralfate and misoprostol [3].

Non-steroidal anti-inflammatory drugs (NSAIDs) including diclofenac are commonly used to treat several disorders like pain, inflammation and fever. However, their use is associated with a relative high incidence of adverse effects in the gastrointestinal (GI) tract. Such damages can form mucosal erosions or ulcers and can occur anywhere along the digestive system from the esophagus to the colon. In the small intestine, the damage can sometimes be found in long-term NSAID users. The greatest concern from a clinical standpoint is the progression of ulcers to the stage of the perforation and the risk of the severe bleeding from ulcers [4].

The ability of NSAIDs like diclofenac to cause ulceration and bleeding in the upper gastrointestinal tract was first documented by the endoscopic study of Douthwaite and Lintott in [5]. The development of safer NSAIDs or effective therapies for the prevention of the adverse reactions of existing NSAIDs requires a better understanding of the pathogenesis of NSAID-induced ulcer disease. NSAIDs cause damage to the gastro-duodenal mucosa via several mechanisms including the topical irritant effect of these drugs on the epithelium, impairment of the barrier properties of the mucosa, suppression of gastric prostaglandin synthesis, reduction of gastric mucosal blood flow and interference with the repair of superfacial injury [6].

The presence of acid in the lumen of the stomach also contributes to the pathogenesis of NSAID induced ulcers and bleeding by impairing the regeneration process interfering with haemostasis and inactivating several growth factors that are critical in the mucosal defense and repair [7].

The main factor that limits NSAID in clinic is the progression of upper gastrointestinal adverse effects including ulcers, complications such as bleeding, and dyspepsia. Strategies had been recommended to decrease GI injury in NSAID users include co-therapy with misoprostol or proton-pump inhibitors and/or use of COX-2 selective inhibitors [8].

Sodium alginate is a water soluble excipient which forms a reticulated structure which can be cross-linked with divalent calcium chloride to form insoluble meshwork. Alginate's unique property of forming water insoluble calcium alginate gel through ionotropic gelation with calcium ions is a simple, mild and eco-friendly condition to encapsulate drugs. Another important property of alginate beads is their re-swelling ability. This property is sensitive to the environmental pH 6. Alginate has a property of coating the drug core and also acts as a release rate retardant [9,10].

Hydroxypropyl methylcellulose (HPMC), a semi-synthetic derivative of cellulose, has its popularity

for the formulation of controlled release (CR) dosage forms as a swell-able and hydrophilic polymer. Its nontoxic property, ease of handling, ease of compression, ability to accommodate a large percent of drug, negligible influence of the processing variables on drug release rates, and relatively simple tablet manufacturing technology make it an excellent carrier material [11].

Pantoprazole is a proton pump inhibitor drug (PPI) drug which is used for short-term treatment of erosion and ulceration of the esophagus caused by gastro esophageal reflux disease [12]. Pantoprazole is a PPI that suppresses the final step in gastric acid production by forming a covalent bond to two sites of the (H^+,K^+)-ATPase enzyme system at the secretory surface of the gastric parietal cells [13].

Fixed NSAID/PPI combinations will likely help to solve the gastrointestinal compliance problem. The first representative role of this group of drugs is treating the signs and symptoms of osteoarthritis (OA), rheumatic arthritis (RA), and ankylosing spondylitis. For decreasing the risk of developing gastric ulcers in patients at risk has just been approved by the FDA [14]. An additional advantage of PPI combination is the lower incidence of heartburn, acid regurgitation, and sleep disturbance. Future guidelines will probably recommend combination of NSAIDs, as well as coxibs with a PPI, as first-line medication for all risk patients [15].

Misoprostol is a synthetic prostaglandin E1 (PGE1) analog that is widely used to prevent gastric ulcers, to treat missed miscarriage, to induce labor, and also to induce abortion [16]. Misoprostol is approved for use in the prevention of NSAIDs induced gastric ulcers. It acts upon gastric parietal cells, inhibiting the secretion of gastric acid via G-protein coupled receptor mediated inhibition of adenylate cyclase, which leads to decreased intracellular cyclic AMP levels and decreased proton pump activity at the apical surface of the parietal cells. Because other classes of drugs, especially H2-receptor antagonists and proton pump inhibitors, are more effective for the treatment of acute peptic ulcers. Misoprostol is only indicated for use by people who are both taking NSAIDs and are at high risk for NSAIDs induced ulcers, including the elderly and people with ulcer complications. Misoprostol is sometimes coprescribed with NSAIDs to prevent their common adverse effects of gastric ulceration (e.g. with diclofenac in 'Arthrotec') [17].

The objective of this study was to compare our prepared formula which contains diclofenac sodium in the form of microbeads in combination with pantoprazole with a marketed formula which contains diclofenac sodium in combination with misoprostol to show the ability of both formulations to decrease the ulcerogenic activity of diclofenac sodium. The ulcerogenic activity and histopathological effects of the prepared formulation were compared with a marked formula containing the drug with misoprostol which orally administered to male Wistar rats.

2. MATERIALS AND METHODS

2.1 Materials

Diclofenac sodium (Sigma- Aldrich, St. Louis, Mo, USA) was a gift sample kindly supplied by Amriya pharmaceutical industries, Alexandria, Egypt. Pantoprazole (Sigma-Aldrich, St. Louis, Mo, USA) was a gift sample kindly supplied by Sigma pharmaceuticals industries, Quweisna, Egypt. Misoprostol (Pfizer, New York, USA) was a gift sample kindly supplied by Sigma pharmaceuticals industries, Quweisna, Egypt. Hydroxypropylmethylcellulose (HPMC) and sodium alginate were purchased from RÖhm Pharma GMBH, Darmstadt (Germany). All other reagents were analytical or pharmaceutical grade and used as received.

2.2 Preparation of Microbeads

Microbeads of diclofenac sodium were prepared by ionotropic gelation technique [18]. The microbeads were prepared in an environment free from organic solvents by dropping a mixture of colloidal copolymer dispersion, the dispersed drug (diclofenac sodium), formed mucilage of sodium alginate in calcium chloride solution, which acted as counter ions. The droplets instantaneously formed gelled spherical beads due to cross-linking of calcium ion with the sodium ion which remain ionized in the solution [19].

Chemical reaction between sodium alginate and calcium chloride to form calcium alginate was utilized for the microencapsulation of diclofenac sodium core material. Preliminary work on the preparation of microbeads revealed that stirring speed and curing time greatly affected the size of microbeads [20,21]. Smaller particles can be prepared by adjusting stirring rate to 500 rpm and curing time for 2 h and also depending upon the height of the syringe of dropping from the

level of counter ion solution as well as compressed force on the plunger of the syringe. The gelled particles were cured to get sufficiently hardened beads, filtered, washed and dried. The colloidal polymer particles fused into the polymer matrix during drying with the drug being dispersed in the latex.

2.2.1 Preparation of alginate-HPMC microbeads

Microbeads were prepared using sodium alginate and HPMC as coating polymers. To 50 ml of deionized water, HPMC was added and stirred with an electric stirrer at 500 rpm to form mucilage. Sodium alginate was added to form uniform dispersion. A weighed quantity of diclofenac sodium was added and homogenized for 5 min. The resulting dispersion was dropped through a syringe with a needle into 100 ml of 5%w/v aqueous calcium chloride solution and stirred at 500 rpm. After stirring for 30 min the formed beads were separated by filtration, washed with distilled water, dried at 70℃ for 6 h in an oven [22].

2.3 Characterization and Evaluation of the Prepared Microbeads

2.3.1 Infrared spectral analysis

The IR spectrum was used to determine the interaction if any of the drug with the polymers used. The infrared spectra of samples were obtained using a spectrophotometer (FTIR, Jusco, Japan). Samples were mixed with potassium bromide (spectroscopic grade) and compressed into discs using hydraulic press before scanning from 4000 to 400 cm^{-1} [23,24].

2.4 *In-vivo* Ulcerogenicity Studies

2.4.1 Experimental animals

Male Wistar rats, weighing 180-200 gm, were obtained from National researches center (Cairo, Egypt).

Rats were maintained at 22±1℃ on a 12h light-dark cycle allowed rat chow and water *ad libitum*. Five groups of rats (n=6 animals per group) were used. The allocation of animals to all groups was randomized. *In-vivo* experimental protocols had the approval of the institutional animal ethics committee (IAEC) (IAEC/PROPOSAL/DB-4/2010).

Before the start of the experiments, rats were housed individually in wire mesh cages to avoid coprophagy under controlled environmental conditions. Food was withdrawn for 36 h but water was allowed *ad libitum* [25]. The absence of ulcers in some of the treated groups has revealed that the pre-fasting conditions alone doesn't induce ulcer. Table 1 shows the experimental design and animal groups.

As described in the studies [26-28] on the morning of the experiments each fasted rat was orally administered 1 ml solution of the assigned drug by oral gavage in a dose equivalent to 10 mg per kg (body weight) of diclofenac [29], 100 µg per kg of misoprostol [30] and 5 mg per kg (body weight) of pantoprazole [31] according to the group type. Magnetic stirring was utilized to obtain a well-dispersed suspension of the drug and the microbeads. Six hours later, each animal was anaesthetized with ether, and the abdomen was opened. This time interval for drug

Table 1. Experimental design and animal groups

Group number	Administered formula	Treatment
I	(Control group)	Rats were orally administered (p. o.) 1 ml distilled water
II	Diclofenac (10 mg/kg)	Rats were administered (p. o.) 1 ml of diclofenac solution
III	Diclofenac (free drug)+ misoprostol (100 µg/kg)	Rats were administered (p. o.) 1 ml of diclofenac (free drug)+ misoprostol
IV	Diclofenac(free drug)+ pantoprazole (5 mg/kg)	Rats were administered (p. o.) 1 ml of diclofenac (free drug)+ pantoprazole (5 mg/kg)
V	Diclofenac-sodium alginate-HPMC microbeads+ pantoprazole	Rats were administered (p. o.) 1 ml diclofenac- sodium alginate -HPMC microbeads+ pantoprazole

administration was subjected to the time table required for induction of peptic ulcer according to Saheed et al. [32] who used Indomethacin as an example of NSAIDs which induced peptic ulcer. The animals were humanely sacrificed by cervical dislocation. The abdomen was opened and the stomach excised. The stomach was thereafter opened along greater curvature and gastric content was drained into a centrifuge tube. Five ml of distilled water was added and the resultant solution was centrifuged at 3000 rpm for 10 min. The supernatant obtained was thereafter used for biochemical analyses. The cleaned stomachs were preserved in 0.1 M phosphate saline buffer (1:4 (w/v), pH 7.4) prior to macroscopic examination [33].

2.4.2 Macroscopic examination of gastric ulcers

The freshly excised stomachs were examined macroscopically for hemorrhagic lesions in the glandular mucosa. Immediately, after the animals were sacrificed.

2.4.2.1 Calculation of ulcer incidence, mean ulcer score, cumulative ulcer length per rat and ulcer index

The percentage of ulcer incidence of animals with gastric ulcer for each group was calculated by (number of rats showing ulcer divided by 6 and multiplied by 100) [34]. The mean ulcer score for each group is calculated by counting the total number of all ulcers in the six rats of each group and divided by 6 [34]. The length (mm) of each lesion was measured [35] and the cumulative ulcer length (mm) per rat for each group is calculated by counting the total length of all ulcers in the six rats of each group and divided by 6 [34]. Mucosal damage was examined and assessed with the help of a 10x binocular magnifier. The severity of mucosal damage was assessed by the modification of a previously reported rating scale [36].

Degrees of ulceration in the indomethacin-treated animals were quantified using the procedure outlined by Szabo and Hollander [37]. Briefly, cleaned stomachs were pinned on a corkboard and ulcers were scored using dissecting microscope with squaregrid eyepiece based on grading on a 0-5 scale. Ulcers of 0.5mm diameter were given a score of 1 mm whereas ulcers of diameters 1 mm and 2 mm were given scores of 2 mm and 4 mm, respectively. Stomach with no pathology was assigned a score of zero.

2.4.3 Histopathological examination of stomach sections

Following Sacrifice of rats, stomach tissues were individually fixed in 10% formalin, dehydrated, paraffin embedded, processed, sectioned in slices with 4 µm thickness and stained with H & E (haematoxylin and eosin). The microscopic and histological scoring was carried out by a pathologist, blind to the treated groups [38]. The tissue sections were examined under an Olympus BX51 (Olympus Corporation, Tokyo, Japan) microscope and images were captured with a digital camera attached to it [39].

2.5 Statistical Analysis

All values are expressed as mean ± S.E. The Tukey test or the Student's t-test for unpaired results was used to evaluate differences between more than three groups or between two groups, respectively. Differences were considered to be significant for values of $P < 0.05$.

3. RESULTS

3.1 Infrared Spectral Analysis

The IR spectra are shown in Fig. 1.

From Fig. 1 it is clear that, the IR spectrum of free drug (diclofenac sodium) (Fig.1-a) shows a characteristic peak at 3386 cm^{-1} due to N-H stretching frequency of secondary amine. The absorption bands at 1305 and 1282 cm^{-1} resulted from C-N stretching and the peaks at 1556 and 1574 cm^{-1} were due to C=C stretching and C=O stretching of carboxylate group, respectively. The C-Cl stretching characteristic peak was observed at 746 cm^{-1}. The IR spectrum of diclofenac sodium alginate-HPMC microbeads (Fig. 1-b) shows all the principal characteristic peaks related to diclofenac sodium without any change in their position, indicating no possibility of chemical interaction between the drug and formulation ingredients.

3.2 Effect of Diclofenac, Its Combination with Misoprostol as well as Its Combination with Pantoprazole on Ulcer Incidence, Mean Ulcer Score Rat and Cumulative Ulcer Length per Rat

A summary of gastric ulcer data for this experiment is shown in Table 2. Only gastric glandular ulcers were observed.

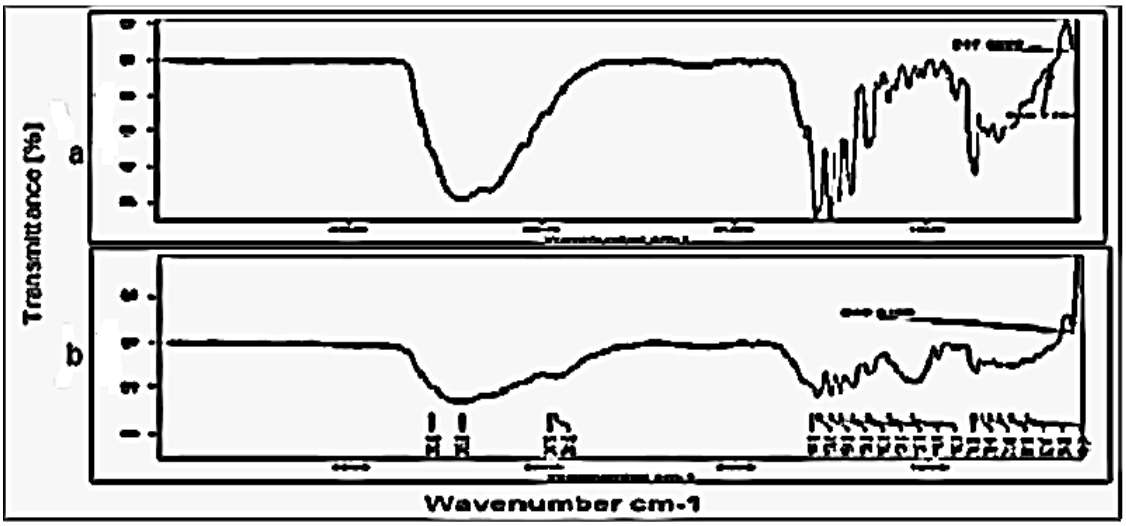

Fig. 1. IR spectra of free drug (diclofenac Sodium) (a), and diclofenac sodium- sodium alginate-HPMC microbeads (b)

Table 2. Effect of diclofenac, its combination with misoprostol as well as its combination with pantoprazole on ulcer incidencemean ulcer score and cumulative ulcer length per rat

Group number	Ulcer incidence		Mean ulcer score	Cumulative ulcer length per rat (mm)
I	0.00%	(0/6)	0.00±0.00	0.00±0.00
II	100%	(6/6)	12.14±0.21	38.19±0.54
III	16.66%	(1/6)	0.18±0.47	0.44±0.37
IV	0.00%	(0/6)	0.00±0.00	0.00±0.00
V	0.00%	(0/6)	0.00±0.00	0.00±0.00

- *Rats were treated as previously described in the experimental design*
- *All data for mean ulcer score is presented as mean ± S.D. (n=6)*

Rats of Group I (control group) which were administered distilled water showed zero ulcer incidence as, no one developed ulcer from the total number of rats of this group (all stomachs are of normal type).

For Group II, in which six rats were administered 10 mg/kg of diclofenac, all the rats showed ulcers in their stomachs with ulcer incidence of 100%, average ulceration number per rat of 10 and an average cumulative ulcer length per rat of 38.54mm were shown.

For Group III, in which six rats were administered diclofenac (10 mg/kg) plus misoprostol (100 µg/kg), only one rat showed ulcer with ulcer incidence of 16.66, average ulceration number per rat of 0.18 mm and an average cumulative ulcer length per rat of 0.44 mm were shown.

For Group IV, in which six rats were administered diclofenac (10 mg/kg) plus pantoprazole (5 mg/kg)] showed zero ulcer incidence as, no one developed ulcer from the total number of rats of this group (all stomachs are of normal type).

For Group V, in which six rats were administered diclofenac-sodium alginate- HPMC microbeads plus pantoprazole showed zero ulcer incidence as, no one developed ulcer from the total number of rats of this group (all stomachs are of normal type).

The obtained results indicate that the combination of diclofenac with either misoprostol or pantoprazole significantly reduced ulcer incidence, number of ulcers per rat and cumulative ulcer length per rat ($p < 0.05$) as compared with the data of diclofenac group in animals administered the same dose of the drug. Microencapsulation of diclofenac using sodium alginate and HPMC significantly reduced gastric irritations and gastric ulcers compared to the free drug ($p<0.05$).

3.3 Effect of Diclofenac, Its Combination with Misoprostol as well as Its Combination with Pantoprazole on Ulcer Index

Based on the severity of mucosal damage, each specimen was assigned a score. The scores were averaged and the mean score was tabulated as the ulcer index for the drug suspension administered. Six determinations were made for each suspension (on all six rats from each individual treatment group). Ulcer index of all animal groups is presented in Table 3.

From the Table 3, it is evident that, Group I (control group) showed ulcer index of zero. Group II; (diclofenac 10 mg/kg) showed ulcer index of 8.31. Group III; diclofenac (free drug) plus misoprostol (100 µg/kg) showed ulcer index of 0.20. Group IV; diclofenac (free drug) plus pantoprazole (5 mg/kg) showed ulcer index of zero. Group V; diclofenac-sodium alginate-HPMC microbeads plus pantoprazole showed ulcer index of zero.

The obtained results indicate that combination of diclofenac with either misoprostol or pantoprazole significantly reduced ulcer index ($p<0.05$) as compared with the data of diclofenac group in a free form in animals administered the same dose of the drug.

The obtained results indicate that, encapsulating the drug in a carrier as well as slow diffusion of the drug into the mucosal media could alleviate the problem of gastric ulceration. Microencapsulation of diclofenac with sodium alginate and HPMC followed by mixing with pantoprazole significantly reduced gastric irritations and gastric ulcers compared to either the free drug or free drug with pantoprazole ($p<0.05$).

3.4 Macroscopic Observation

Macroscopic examination of rat stomachs of the control group administered distilled water and rat stomachs which were administered diclofenac, its combination with misoprostol as well as its combination with pantoprazole is presented in Fig. 2.

From Fig. 2 it is obvious that, gross study of gastric lumina of the control group showed completely an apparent normal gastric mucosa regarding a normal rauga and mucous covering layer (Fig. 2a).

A rat stomach which administered a dose of (10 mg/kg) of diclofenac, showed pin point hemorrhagic area as well as a wide spread hemorrhaging as indicated by the red spots which are blood clots (Fig. 2b).

A rat stomach which administered diclofenac (free drug) plus misoprostol (100 µg/kg) showed normal gastric mucosa with evoked a focal area of congestion (Fig. 2c).

A rat stomach which administered diclofenac (free drug) plus pantoprazole (5 mg/kg) showed a normal gastric mucosa with a small area of congestion (Fig. 2d).

A rat stomach which administered diclofenac-sodium alginate-HPMC microbeads plus pantoprazole showed an apparently normal gastric mucosa (Fig. 2e).

3.5 Histolopathological Examination

The histopathological pattern of the mucosal specimens was studied by examining the histology of the treated and control samples.

Effect of diclofenac, its combination with misoprostol as well as its combination with pantoprazole on stomach tissue histopathology is presented in Fig. 3.

Histopathological examination of Hx & E stained stomach sections of distilled water administered control rats (n=6), revealed that all the six animals showed completely normal gastric mucosa with excess mucous layer (Fig. 3a).

Table 3. Effect of diclofenac, its combination with misoprostol as well as its combination with pantoprazole on ulcer index

Treatment	Ulcer index
Group I (Control group)	0.00±0.00
Group II Diclofenac (10 mg/kg)	8.31±0.45
Group III Diclofenac (free drug)+ misoprostol (100 µg/kg)	0.20±0.87
Group IV Diclofenac(free drug)+ pantoprazole (5 mg/kg)	0.00±0.00
Group V Diclofenac-sodium alginate-HPMC microbeads+ pantoprazole	0.00±0.00

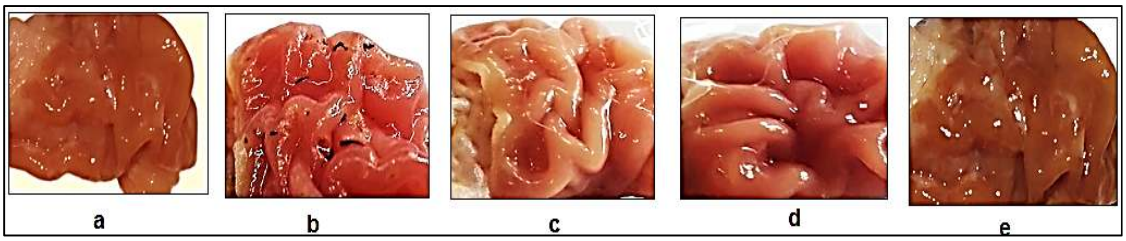

Fig. 2. Representative images showing morphological changes in rat gastric tissues after administration of diclofenac, its combination with misoprostol as well as its combination with pantoprazole

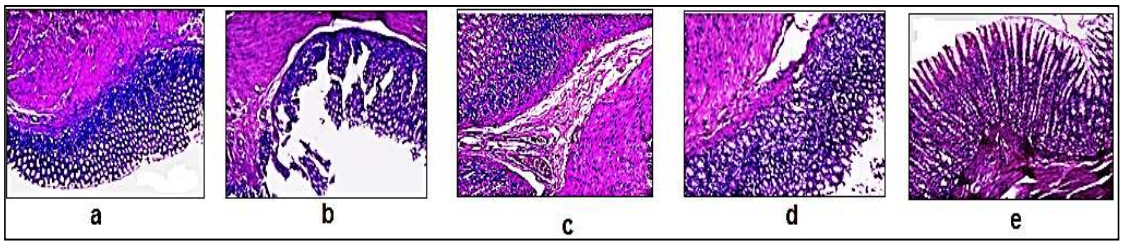

Fig. 3. Representative images showing histological observations in rat gastric tissues after administration of diclofenac, its combination with misoprostol as well as its combination with pantoprazole

In diclofenac (10 mg/kg) administered rats (n=6), histopathological examination revealed that all the six animals showed pronounced necrotic gastric mucosa with severe dilated congested blood vessels in the lamina properia with severe edema infiltrated by inflammatory cells (neutrophil infiltration), also superficial mucosal layer showed marked congestion, necrosis (Fig. 3b).

In diclofenac (free drug) plus misoprostol (100 µg/kg) administered rats (n=6), histopathological examination revealed that stomach tissue of animals showed sub-mucosal congestion and mild inflammation (Fig. 3c).

In diclofenac (free drug) plus pantoprazole (5 mg/kg) administered rats (n=6), histopathological examination revealed that stomach tissue of animals showed mild inflamed gastric mucosa (Fig. 3d).

In diclofenac-sodium alginate-HPMC microbeads plus pantoprazole administered rats (n=6), histopathological examination revealed that stomach tissue of animals showed normal gastric mucosa (Fig. 3e).

4. DISCUSSION

From the obtained results it is clear that, using diclofenac in a dose of (10 mg/kg) produces the largest gastric damage. The ulcer index, the mean ulcer score, cumulative ulcer length per rat as well as ulcer incidence were larger compared with other groups either in case of its combination with misoprostol or in case of its combination with pantoprazole ($p< 0.05$).

After administration of a single dose of diclofenac (free drug) plus pantoprazole (5 mg/kg) formula, significant reduction of 83.34% in ulcerogenic activity as compared to free drug of the same dose in regard to the ulcer index of both groups occurred.

In case of administration of a single dose of diclofenac (free drug) plus pantoprazole (5 mg/kg) and diclofenac- sodium alginate-HPMC microbeads plus pantoprazole formula, significant reduction of 100% in ulcerogenic activity as compared to free drug of the same dose in regard to the ulcer index of both groups occurred.

At acidic pH the alginate beads shrink due to tightening of the gel network, resulting in decreasing drug release from microbeads. The polymer is eroded at alkaline pH and the contents of microbeads are released in a controlled manner by both diffusion and slow erosion of polymer matrix. Therefore, one can assume that the diclofenac sodium microbeads

are promising pharmaceutical dosage forms by providing controlled release drug delivery systems and avoiding the dose related side effects in the entire physiological region [18]. Our study agrees with the reports of Zein et al., who confirmed that microencapsulation of diclofenac by sodium alginate-HPMC played a great role in preventing gastric ulceration caused by diclofenac [40].

The integrity of the gastric mucosa depends on the balance between aggressive (HCl, pepsine) and protective factors (mucus and HCO_3- secretion, prostaglandins, mucosal blood flow, nitric oxide) [41]. The treatment is effective depending not only on the blockade of acid secretion, but also on the increased production of factors responsible for protecting the gastric mucosa, thus avoiding damage to the epithelium [42].

Inhibition of prostaglandin synthesis is well recognized as the central mechanism by which gastrointestinal injury occurs [43]. This is a result of inhibition of cyclooxygenase enzyme which converts unsaturated fatty acids (which are released during cell injury) such as arachidonic acid to prostaglandins. In the stomach, prostaglandin synthesis is protective as a result of enhanced mucosal blood flow and stimulation of mucous and bicarbonate secretion [44].

In contrast, in arthritis, prostaglandins mediate pain and some components of inflammation. Recognition of two isoforms of cyclooxygenase, with COX-1-predominating in the stomach and an inducible COX-2 expressed at sites of inflammation offer the prospect of separating the beneficial effects of inhibiting prostaglandin synthesis in joints from the harmful effects of inhibiting it in the stomach [45].

In the present study, the significant increase in ulcer index following oral administration of diclofenac in the ulcerated rats may be attributed to either free radicals formation or inhibition of prostaglandin synthesis. Decreased prostaglandin level has been attributed to impaired gastro-protection and increased gastric acid secretion which are important events in the etiology of mucosal ulceration. Our study agrees with the reports of Bech et al. [46], Biplab et al. [47] and Muhammed et al. [48] where diclofenac was reported to have caused alterations in gastric secretions of rats.

The primary objective of the present investigation was to determine whether the combination of diclofenac with either misoprostol or pantoprazole provides protection against diclofenac -induced damage to gastric mucosa. Results showed that both combinations of diclofenac with misoprostol or pantoprazole used in this study are capable of providing protection to the gastric mucosa against diclofenac -induced gastric injury. In most of our experiments, the diclofenac -induced gastric ulceration was maximally protected by coating the drug with enteric-polymers (HPMC) then combining it with pantoprazole at the dose of 5mg/kg (fed orally).

Our results comply with previous reports, where pantoprazole was found to enhance healing of ulcers due to its potent anti-secretory effect. In addition to anti-secretory activity higher protective effect of pantoprazole may be due to its gastric activity also. It has already been shown that repeated treatment with proton pump inhibitors increase the level of PGs in the gastric mucosa, which is considered to be chief mediators in gastric cyto-protection. Our results agree with the reports of Kawano et al. [49], Okabe et al. [50] and Ruwart et al. [51].

Previous reports also indicate the quicker healing effect of omeprazole than misoprostol is because proton pump inhibitors directly inhibit acid secretion [52]. Role of PGs alone may not be sufficient in accelerating the healing process as is evident from lesser efficacy of misoprostol. Even though misoprostol accelerated healing process more than other agents but its effect is less than that of pantoprazole. Protective effect of misoprostol is due to its prevalent direct cytoprotective effect of PGs coupled with anti-secretory effect [53,54].

The histological studies showed that, the reduction of ulceration is evident from the macroscopic as well as microscopic studies which showed a complete protection of the tissue morphology with no ulcers was observed, indicating again the effectiveness of these combinations against diclofenac -induced gastric ulceration in rats.

5. CONCLUSION

The obtained results indicated that coating diclofenac with sodium alginate and HPMC and then combination with pantoprazole offers a good opportunity for controlling drug release as well as playing an important role in protecting the GIT from hemorrhage and ulceration generally

induced by NSAIDs. The proposed formulation showed better protection of the stomach compared with the marketed formula. FT-IR studies revealed that there wasn't any significant drug interaction between the drug and the polymers which were used in coating the drug (sodium alginate and HPMC). It is clear that the major contribution of the local ulcerogenic effects of diclofenac can be appreciated from the decreased incidence and magnitude of ulcers following the use of enteric coated formula using sodium alginate and HPMC polymer and its combination with pantoprazole which gave the best results compared with the other formulae. It is possible to overcome the problem of gastric damage during the use of diclofenac, by avoiding the exposure of the drug to the ulcer-prone area of the GI tract.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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