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Protection by gabapentin against indomethacin and ethanol-induced gastric mucosal damage in the rat. A histological and histochemical study

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ABSTRACT

Gabapentin is an antiepileptic drug which is widely used to treat chronic neuropathic pain. In this study, the effect of gabapentin on gastric mucosal damage induced in the rat by indomethacin or 96% ethanol was examined. Rats were treated with indomethacin at a dose of 20 mg/kg subcutaneously (s.c.) for two consecutive days or 96% ethanol (1 ml, intragastrically) alone or in combination with the gabapentin at doses of 25, 50 or 100 mg/kg, given intraperitoneally (i.p.). Rats were euthanized 48 hr after indomethacin or 1h after ethanol administration when stomachs were removed, opened and subjected to histological and histochemical investigation. Results showed that indomethacin caused severe exfoliation of the gastric epithelial cells as well as disruption of mucosal layer of stomach. The intragastric administration of 96% ethanol caused massive destruction of the upper two thirds of the gastric mucosa and marked vascular dilatation and congestion. Either ulcerogenic agent resulted in markedly decreased in the normal distribution of the neutral mucopolysaccharides assessed by Periodic acid Schiff's (PAS) stain staining. Gabapentin given at doses of 50 or 100 mg/kg conferred remarkable and dose-dependent protective activity against gastric mucosal damage caused by indomethacin or 96% ethanol. Also, the distribution and magnitude of the PAS positive reaction were essentially normal following the administration of gabapentin at a dose of 100 mg. These findings imply that gabapentin protects against ethanol or non-steroidal anti-inflammatory drug-induced damage to the gastric mucosa.

Keywords: Gabapentin; gastric ulcer; non-steroidal anti-inflammatory drugs; gastric mucosa; indomethacin; prostaglandins; cyclooxygenase

1. INTRODUCTION

The gastric mucosa is constantly exposed to injurious agents that can damage the gastric mucosal barrier and breach the mucosa, resulting in gastric erosions or ulceration. Apart from gastric acid and pepsins secreted by the gastric mucosa, the use of non-steroidal anti-inflammatory drugs and ethanol are two main

exogenous factors that damage the gastric mucosa (Werther, 2000). Non-steroidal anti-inflammatory drugs (NSAIDs) constitute one of the most widely prescribed agents in clinical practice, which are used in the treatment of fever, headache, inflammatory and painful arthritic conditions such as rheumatic arthritis, acute gout, rheumatoid disease, traumatic injuries, post-operative pain and bursitis (Bacchi et al., 2012). Their use has been associated with the development of acute gastric erosions, acute and chronic ulcers and bleeding which may be potentially serious and endangers life (Hawkey, 2000; Russell, 2001; Rainsford et al., 2007). NSAIDs which act by inhibition of the cyclo-oxygenase enzyme (COX), the rate limiting step in production of cytoprotective prostaglandins are those who are likely to cause gastric mucosal injury (Vane et al., 1998). There are two forms of the enzyme, a constitutively expressed COX-1 endued with the synthesis of prostaglandins involved in gastroduodenal mucosal protection and an inducible COX-2 whose expression is increased in inflammatory conditions (Vane et al., 1998; Botting, 2006). The inhibition of prostaglandins synthesis results in impairment of such gastric mucosal protective mechanisms as mucosal blood flow, mucus/bicarbonate secretion, surfactant phospholipid and epithelial proliferation and hence expose the gastric mucosa to luminal acid and other potentially damaging agents (Shiotani and Graham, 2002). The ethanol-induced gastric mucosal injury, on the other hand, is caused by a vascular mechanism, in which the microvasculature is largely affected and damage is rapidly developing minutes after the ethanol is instilled into the stomach (Szabo et al., 1985; Tarnawski et al., 1985).

Gabapentin, an analogue of gamma aminobutyric acid (GABA) is widely used antiepileptic drug which recently found extended application in neuropathic pain syndromes such as that occurring in diabetes mellitus, post-herpetic neuralgia, trigeminal neuralgia besides pain caused by fibromyalgia, disc herniation and after surgery (Gilron and Flatters, 2006; Schmidt et al., 2013). The drug is therefore likely to be used in conjunction with NSAIDs to alleviate pain. In the present study, we therefore, aimed to investigate the effect of the gabapentinoid drug gabapentin on gastric mucosal lesions induced in rats by the NSAID indomethacin, a non-selective COX inhibitor or ethanol, using histopathological and histochemical approaches.

2. MATERIALS AND METHODS

Animals

Sprague-Dawley rats of either sex, weighing 170-180 g, provided by the Animal House of the National Research Centre, Cairo, were used in the study. Rats were group-housed under-temperature and light-controlled conditions and allowed standard laboratory rodent chow and tap water ad libitum. The experimental studies were done according to the recommendations of the Ethics Committee of the National Research Centre and the Guide for Care and Use of Laboratory Animals by the US National Institutes of Health (Publication No. 85-23, revised 1996).

Drugs and chemicals

Gabapentin (Delta Pharma, Cairo, Egypt) and indomethacin (Kahira Pharm & Chem. IND Co., Cairo, Egypt) were used in the study. Gabapentin was dissolved in isotonic saline solution (0.9% NaCl) immediately before use. Indomethacin was dissolved in 5% solution of sodium bicarbonate. The rest of chemicals and reagents used were purchased from Sigma (St Louis, MO, USA) and were of analytical grade.

Gastric ulcerogenic studies

Indomethacin-induced gastric mucosal damage

Gastric mucosal damage was induced by subcutaneous indomethacin administered at a dose of 20 mg/kg for two successive days. The effects of gabapentin at doses of 25, 50, or 100 mg/kg, i.p., administered at the same time as indomethacin injection were investigated. The vehicle was used on the control group. The rats were given unlimited access to food and tap water and euthanized 24 hours after the final treatment.

Ethanol-induced gastric mucosal damage

Rats were fasted for 18 h but had free access to tap water. Gabapentin at doses of 25, 50 or 100 mg/kg was given i.p., 30 min prior to ethanol (96%, 1 ml, orally). The stomachs were then removed and opened along the greater curvature, rinsed with saline and then with formal saline to remove any food contents in them.

Gastric histological and histochemical studies

The stomach specimens were preserved in 10% neutral-buffered formal saline for at least for 72 hours. Specimens were cleaned in xylene, washed with tap water for 30 minutes, dehydrated in ascending grades of alcohol and then embedded in paraffin. For the

histopathological research, serial sections of 6 m thick were cut and stained with haematoxylin and eosin (Hx & E) (Drury and Walligton, 1980) or with Periodic acid-Schiff stain (PAS) (McManus and Mowry, 1964) for neutral mucopolysaccharides. Version 8.0 of Adobe Photoshop was used to capture the images.

3. RESULTS

Indomethacin-induced gastric mucosal damage

Haematoxylin and Eosin staining

The gastric mucosa in saline control rats had a normal histology (Figure 1A). The fundic stomach of the rats receiving indomethacin showed macroscopically discernible gastric hemorrhagic lesions. Histological inspection of the tissue indicated that indomethacin caused exfoliation of the gastric epithelial cells along with disruption of mucosal layer of stomach compared with that of control. Ulcer re-epithelialization with an appearance of intact mucosal layer was observed in healed tissue. As shown in figures 1C and 1D, indomethacin had an adverse effect on the mucosa of the stomach, causing the development of ulcerative lesions, a noticeable deformation of the normal structure of the gastric mucosa, atrophy of the majority of the cells in this layer, as well as observable thickening of the muscularis mucosa and dilation of blood vessels.

The effects of indomethacin on the stomach mucosa were dose-dependently mitigated by gabapentin treatment. Although ulcerative lesions showed evidence of regeneration after being treated with gabapentin at a dose of 25 mg/kg, atrophy, particularly in the surface epithelium, and an increase in the thickness of the muscularis mucosa persisted (Figure 2A & 2B). When gabapentin was administered at a dose of 50 mg/kg, the gastric mucosa's normal architecture was restored, and part of the surface epithelial cells once again regained its normal appearance. Nevertheless, muscularis mucosa layer thickening and dilated blood vessels persisted (Figure 2C & 2D). The indomethacin-induced damage was significantly improved by the higher dose of gabapentin (100 mg/kg), which resulted in a notable reduction in muscularis thickness, the disappearance of epithelial cell atrophy and cellular infiltrates and a nearly normalisation of the structure of the gastric mucosa (Figure 2E & 2F).

Periodic acid-Schiff staining

In sections from the saline control group, the neutral mucopolysaccharides that cause a positive reaction (red colour) with PAS stain were distributed normally (Figure 3A). Contrarily, a significant reduction in PAS staining was seen following indomethacin therapy (Figure 3B) and this reduction persisted even after the combination of indomethacin and gabapentin at a dose of 25 mg/kg (Figure 3C). Sections from rats given 50 mg/kg doses of gabapentin and indomethacin demonstrated a positive reaction to the PAS stain that extended to the neck region at the locations of the mucous neck cells (Figure 3D). The amount and distribution of the positive PAS stain result were essentially normalised when 100 mg of gabapentin was administered (Figure 3E & 3F).

Ethanol-induced gastric mucosal damage

Haematoxylin and Eosin staining

The normal histologic appearance of the gastric mucosa in the saline controls is shown in Figure 4A. On the other hand, ethanol caused a severe direct effect on the gastric mucosa, represented by massive destruction of the upper two thirds of the gastric mucosa as well as marked dilatation and congestion of blood capillaries at the site of damage (Figure 4B). Treatment with gabapentin reduced the damaging effects of ethanol in a dose-dependent manner Given at a dose of 25 mg/kg, gabapentin did not significantly lessen the damage, as the ulceration's site could still be made out and detached, deteriorated cells could still be seen (Figure 1, C). Given at doses of 50 or 100 mg/kg, gabapentin aided in the regeneration of gastric mucosal cells, particularly at the ulcer's base (Figure 4D & 4E). The highest gabapentin dose resulted in the greatest regeneration of the upper mucous cell layer (Figure 4F).

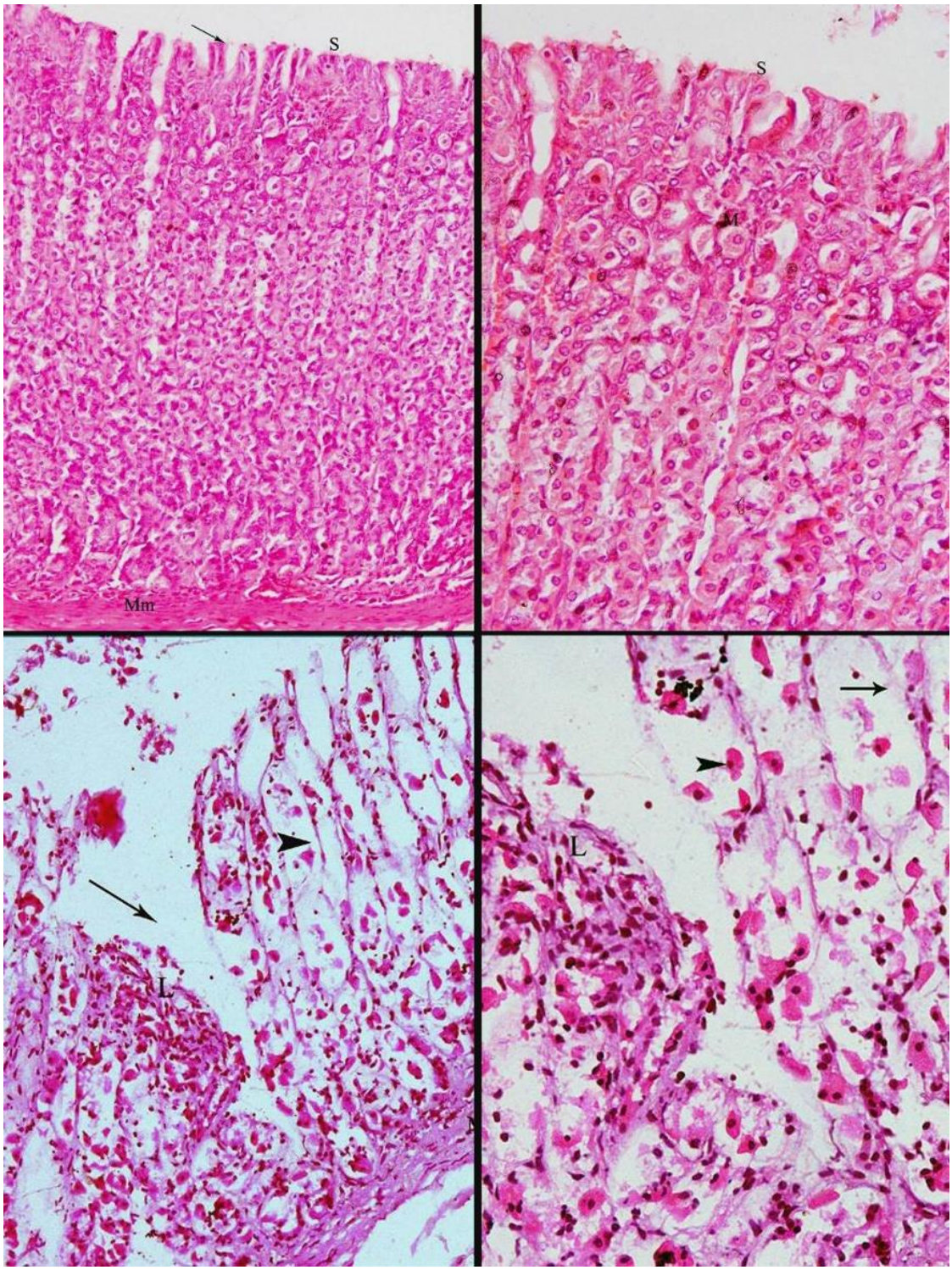

Figure 1 Photomicrographs of sections of rat gastric mucosa after treatment with: (A & B) Saline shows the fundic tubular glands separated from the submucosa by a thin layer of muscularis mucosa, the surface mucous cells and mucous neck cells. (C) Indomethacin only shows severe destruction of an area of the mucosa leading to the formation of ulcerative lesion (arrow). Fibrous tissue with cellular infiltrates (L) atrophied cells (arrow head) making the lumen of the fundic glands appear empty and the pits of the glands appear wider and deeper than normal. The muscularis mucosa layer (mm) shows obvious thickening. (D) A magnification of the previous section shows atrophied scattered parietal cells (arrow head) and other types of gastric gland cells (arrow) (Hx. & E. X 200, 400).

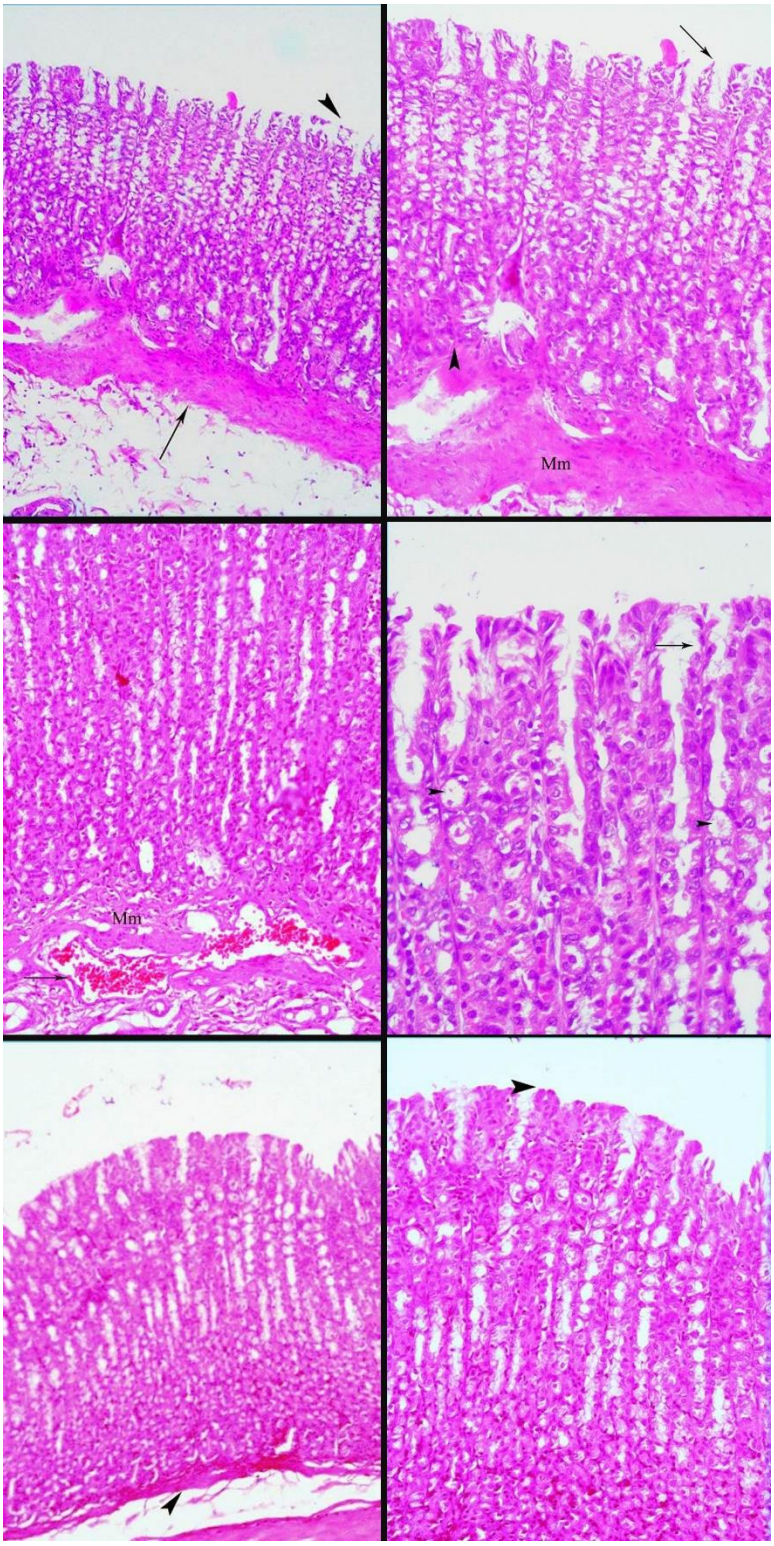

Figure 2 Photomicrograph of sections of rat gastric mucosa treated with: (A) Indomethacin and gabapentin 25 mg/kg: Show the surface epithelial cells are still atrophied (arrow head), the layer of the muscularis mucosa shows marked thickening (arrow). (B) A magnification of the previous section showing cells at the regions of neck and isthmus with vacuolar degeneration and fibrosis with cellular infiltrates (arrow head). (C) Indomethacin and gabapentin 50 mg/kg show restoration of the normal architecture of the gastric mucosa in most parts, although there is dilatation and congestion of supplying blood vessels (arrow) and thickening of muscularis mucosa layer (mm). (D) A magnification of the previous section shows some of the surface cells are still atrophied (arrow), some cells show variable degrees of degeneration (arrow head). (E) Indomethacin and gabapentin 100 mg/kg show normal gastric mucosa and muscularis mucosa layer (arrow head). (F) A magnification of the previous section shows normal surface cells (arrow head) and pits of glands (Hx. & E. X 100 & 200, D X400).

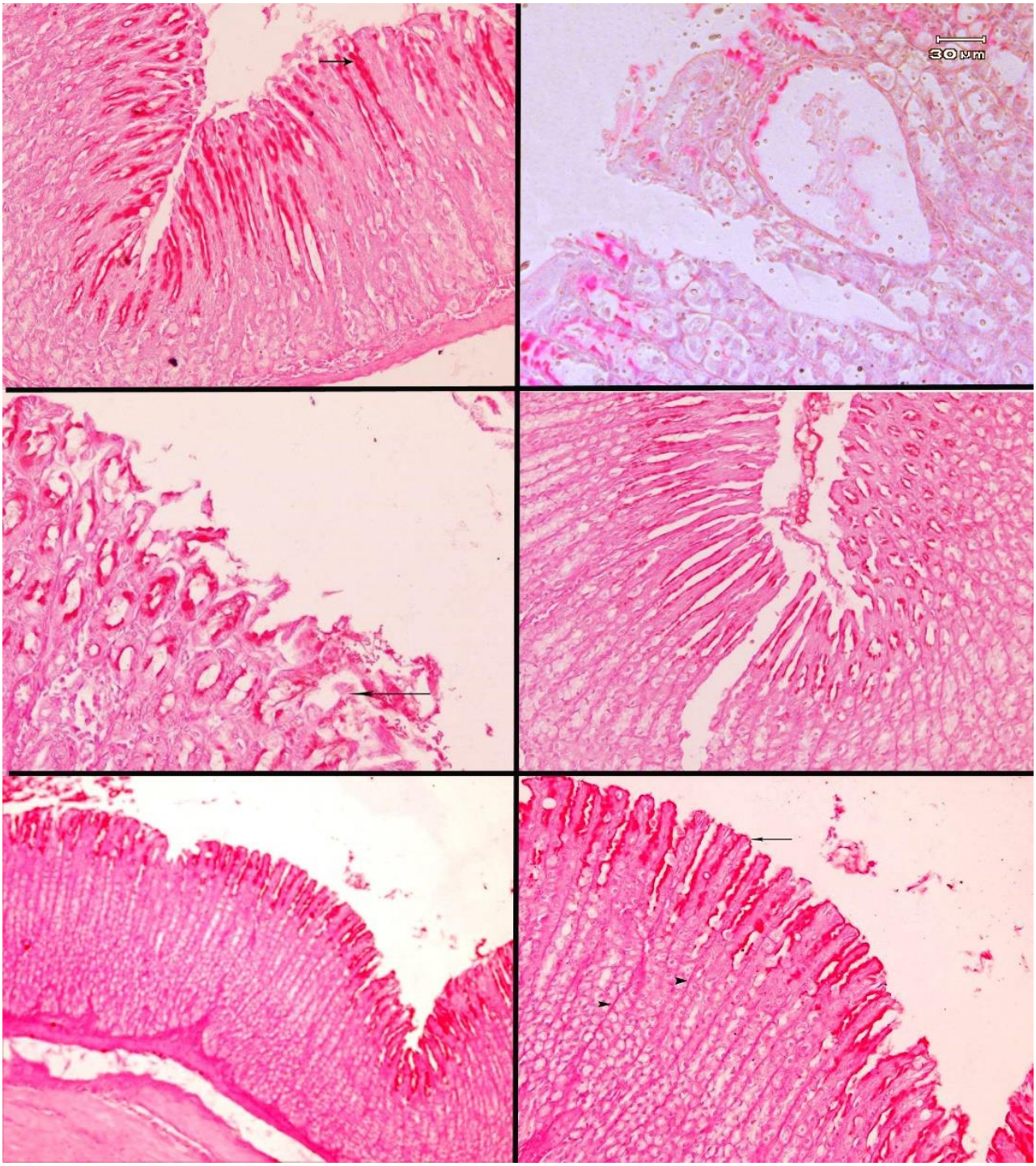

Figure 3 Photomicrograph of sections of rat gastric mucosa attained with PAS after treatment with: (A) Saline shows the normal distribution of the neutral mucopolysaccharides that gives a positive reaction (red color) with PAS stain. (B) Indomethacin shows a remarkable loss of positive reaction for the stain at the ulceration site. (C) Indomethacin and gabapentin at a dose of 25 mg/kg show that there is still a decrease of the positive reaction of the PAS stain in a wide area with exfoliation of the surface epithelial cells. (D) Indomethacin and gabapentin at a dose of 50 mg/kg show the positive reaction of the PAS stain extending in the neck region at the sites of the mucous neck cells. (E) Indomethacin and gabapentin at a dose of 100 mg/kg show nearly normalization of the distribution and amount of the positive reaction of PAS stain. (F) A magnified photomicrograph of the previous section shows the positive reaction of PAS covering the surface and lining the pits of the glands in a continuous layer (arrow). It is also observed in the basement membranes of the capillaries between the fundic glands (PAS X100 for E, X 200 for A, D & F, X 400 for B).

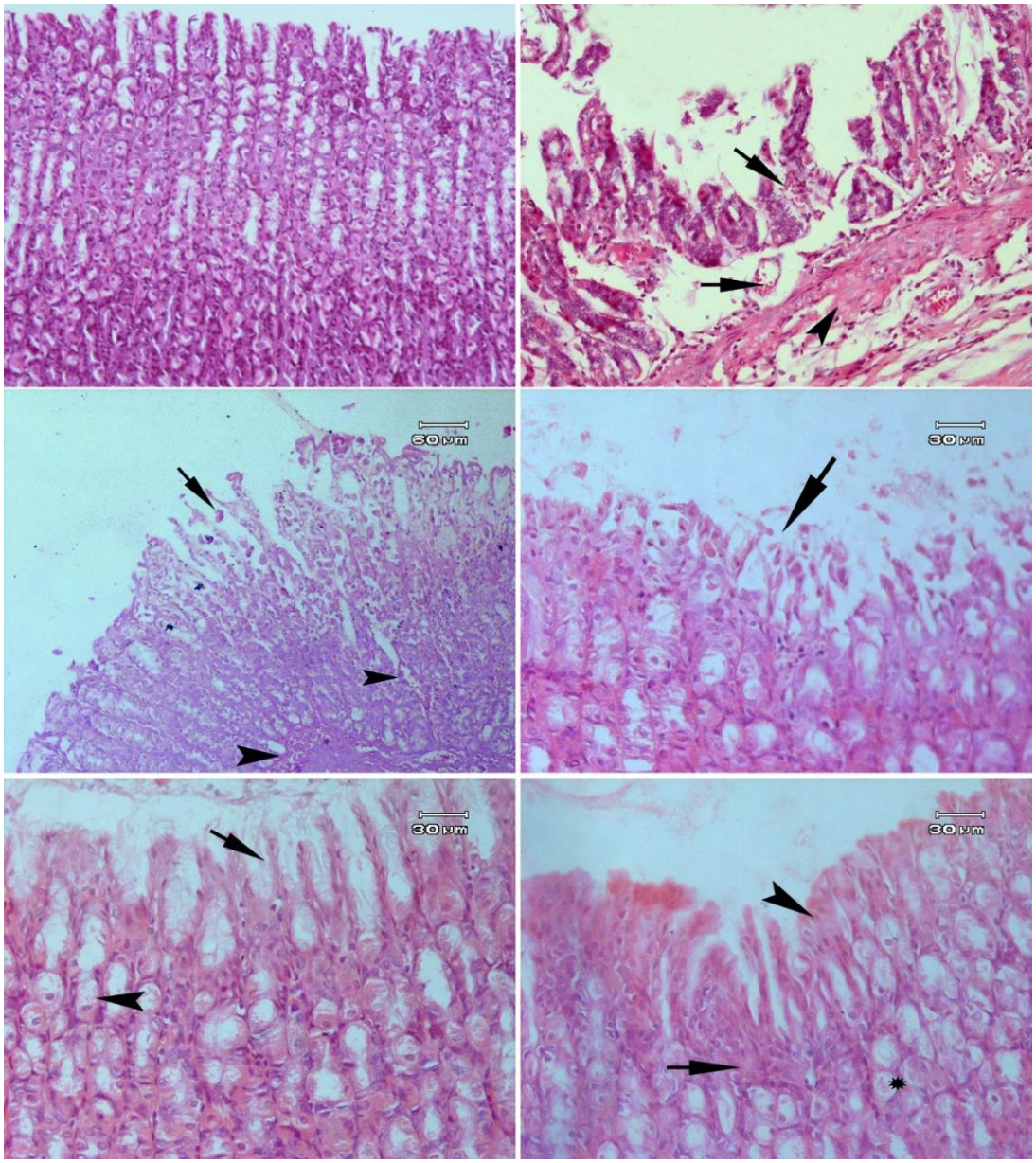

Figure 4 Photomicrographs of gastric mucosa sections stained with Hx & E from: (A) Saline shows the normal structure of the tubular gastric fundic glands. (B) Ethanol only shows massive destruction of the upper two thirds of the gastric mucosa with shedding out of the degenerated cells, dilatation and congestion of blood capillaries (arrow) and marked thickening of muscularis mucosa (arrowhead). (C) Ethanol and gabapentin at 25 mg/kg show degeneration and detachment of gastric glands' cells at the ulceration site (arrow) leaving empty spaces at the site. Deep, deformation of the normal structure of gastric glands is observed as well as dilatation of blood capillaries (arrowhead). (D) Ethanol and gabapentin at 50 mg/kg show restoration of the lower part of the gastric glands, where mucous neck cells and parietal cells appear normal. The upper part of the glands still shows atrophied and degenerated upper mucous cells. (E) Ethanol and gabapentin at 100 mg/kg show normal mucous neck cells in the lower part of the glands (arrowhead). The upper mucous neck cells show slight amelioration, although they are still smaller than normal making the pits of the glands wider than usual (arrow). (F) Ethanol and gabapentin at 100 mg/kg show marked amelioration of the upper mucous cells (arrowhead). Leucocyte infiltration is detected at the base of regenerated ulceration site (arrow).

Periodic acid-Schiff staining

The results obtained from the investigation for mucopolysaccharides by using PAS stain confirmed those of Hx & E examination. In contrast to the saline controls' typical distribution of PAS stain (Figure 5A), ethanol completely deleted mucopolysaccharides at the ulceration site (Figure 5B), which gabapentin gradually and dose-dependently restored (Figure 5C, 5D & 5F).

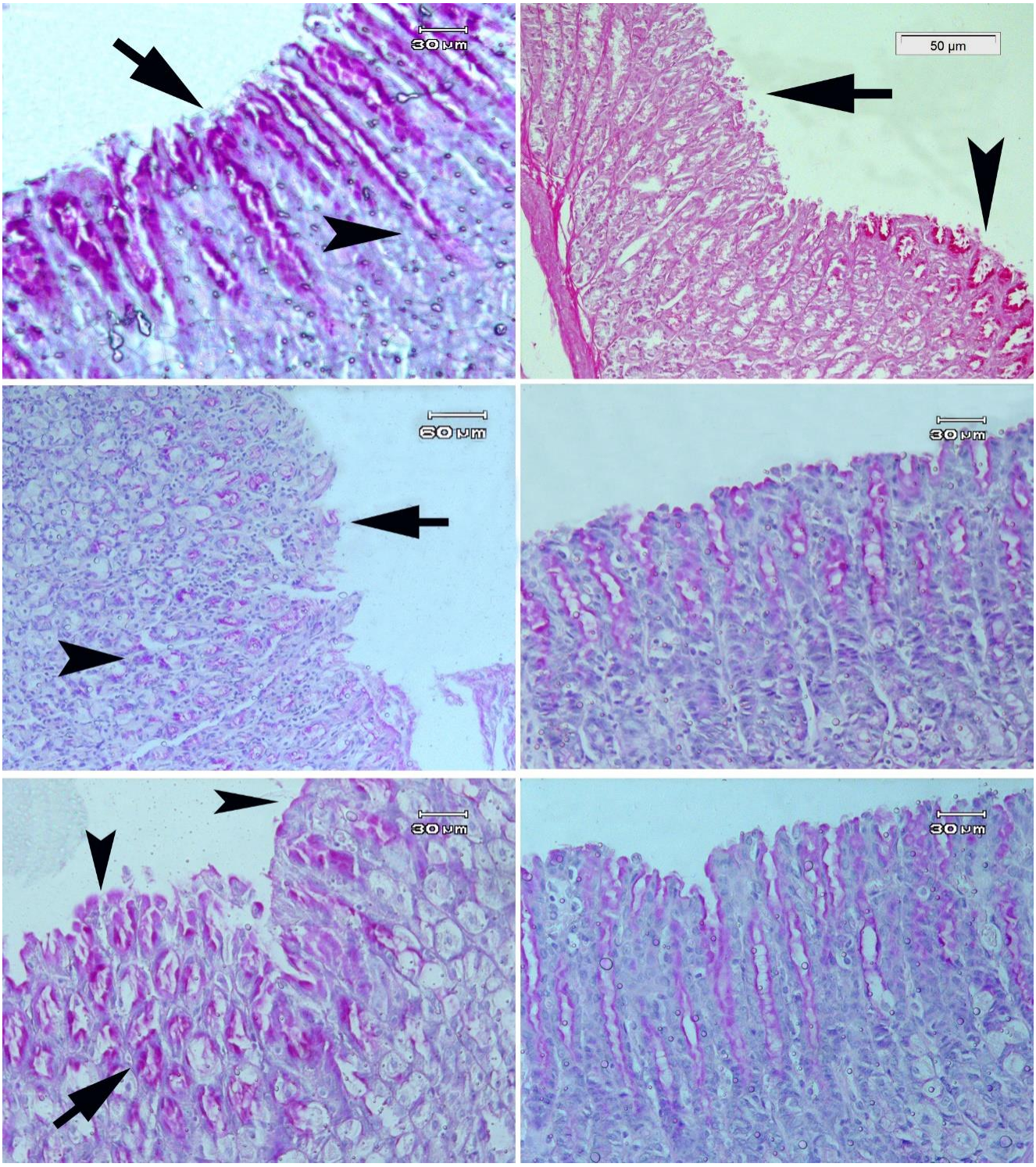

Figure 5 Photomicrograph of gastric mucosa sections stained with PAS from: (A) Saline control rat shows strong positive reaction to the stain on the surface in the upper mucous cells (arrow) and along the tubular gastric glands in the mucous neck cells (arrowhead). (B) Ethanol only shows negative reaction to the stain at the ulcer site (arrow) if compared to the positively reacted area beside the ulcer (arrowhead). (C) Ethanol and gabapentin at 25 mg/kg show that the positive reaction to the stain is still absent (arrow), while in the lower part of gastric mucosa a faint positive reaction to the stain is observed in the mucous neck cells (arrowhead). (D) Ethanol and gabapentin at 50 mg/kg show a good positive reaction to the stain is noticed in the neck region of gastric glands, while at the upper surface the reaction is still weak. (E) Ethanol and gabapentin at 100 mg/kg show a well identified positive reaction to the stain in mucous neck cells (arrow). A thin visible positive reaction is detected on the upper surface of mucosa (arrowhead). (F) Ethanol and gabapentin at 100 mg/kg show normal distribution of the positive reaction to the stain although it is weaker than normal.

4. DISCUSSION

The findings in this study provided the evidence that gabapentin, a drug used in treatment of epilepsy and neuropathic pain conditions exerts gastric protective effects. Gabapentin prevented the development of gastric mucosal damage evoked by the NSAID indomethacin or 96% ethanol. Our histological study showed that indomethacin caused severe damage to the surface epithelial cells and mucosa resulting in an ulcerative lesion. On the other hand, the intragastric administration of ethanol resulted in massive destruction of the upper two thirds of the gastric mucosa together with marked congestion. In both types of mucosal injury, gabapentin at the dose of 50 or 100 mg/kg resulted in remarkable protection of the mucosa, with the effect being a dose-dependent one. The histochemical investigation using PAS reaction revealed the decrease in staining after either indomethacin or ethanol, indicative of cell damage/loss and metabolic perturbations by both ulcerogens. The loss of PAS staining was reversed dose-dependently by gabapentin at 50 or 100 mg/kg.

It is widely accepted that gastric ulcer occurs because of an imbalance between mucosal aggressive factors and mucosal defensive mechanisms (Werther, 2000). NSAIDs and ethanol represents noxious exogenous agents capable of breaking the gastric mucosal barrier and inflicting mucosal damage (Prichard et al., 1988; Wallace, 1997; Knoll et al., 1998). The use of NSAIDs has been shown to increase the risk of upper and lower gastrointestinal bleeding (Lanas et al., 2015; Melcarne et al., 2016). The drugs are broadly divided into two main categories, non-selective NSAIDs and selective COX-2 inhibitors. While COX-1 is the "housekeeping" iso form that is responsible for the synthesis of prostaglandins such as PGE_2 and PGI_2 involved in protecting the gastric mucosa, regulation of renal blood flow and platelet aggregation. COX-2 is induced in inflammatory conditions in response to cytokines and is involved in pain and fever (Vane et al., 1998; Botting, 2006). Aspirin and indomethacin, which are the most potent inhibitors of COX-1 are the two NSAIDs which cause the most damage to the stomach (Vane, 1996). NDAIDs that are non-selective inhibitors of both COX-1 and COX-2 evoke gastric mucosal damage by depriving the mucosa of cytoprotective prostaglandins. This will result in increased gastric acid secretion by parietal cells, decreased mucus and bicarbonate secretion, epithelial cell restitution, surface active phospholipids and mucosal blood flow (Schoen and Vender, 1989; Abdel-Salam et al., 2001; Gyires, 2005).

In support of this notion is the ability of misoprostol, a synthetic PG_E analogue to prevent gastroduodenal ulceration (Graham et al., 1988; Bardhan et al., 1993). NDAIDs may also impair mucosal defenses via mechanisms that involves increase reactive oxygen species (Nam et al., 2012; Ellithey et al., 2019) and impairment of mitochondrial function and depletion of cellular energy (Matsui et al., 2011; Bjarnason et al., 2018). These agents can cause vascular endothelial injury mediated by neutrophils that gain access into the gastric microcirculation (Rainsford, 1983; Wallace, 1990) and also by increasing gastric motility as in case of indomethacin, resulting in microcirculatory perturbations and cell damage (Takeuchi et al., 1996). NSAID-induced gastropathy and ulcers are also dependent on the presence of gastric acid and these heal effectively when administering proton pump inhibitors to patients who require continued therapy with NSAIDs (Lazzaroni and Porro, 2009; Melcarne et al., 2016). Indomethacin increases gastric acid secretion in humans and research animals, which could be due in part to the loss of the antisecretory effect of mucosal prostaglandins (Levine and Schwartzel, 1984; Bjarnason et al., 2018). Ethanol is also a known injurious agent to the gastric mucosa which causes gastric hemorrhagic injury through a direct vascular mechanism. Ethanol immediately and markedly constricts submucosal venules, which is followed by capillary congestion and the emergence of extensive gross mucosal haemorrhagic lesions (Yonei and Guth, 1991).

According to studies by Szabo et al., (1985) and Pihan et al., (1986), ethanol can cause vascular damage as soon as 1 to 5 minutes after being instilled into the stomach of rats. This damage may be caused by neutrophils through endothelial cell damage (Kvietys et al., 1990). In addition, gastric mucosa of 96% ethanol-treated rats showed an increase in the proinflammatory cytokines tumour necrosis factor-α and interleukin-1β, suggesting that ethanol-induced damage to the gastric mucosa was caused by an inflammatory process (Ellithey et al., 2019). Hence, it is not surprising that vasodilator drugs, such as capsaicin-type agents, could reduce stomach mucosal lesions brought on by NSAIDs or ethanol (Abdel-Salam et al., 1997, 1999). In the present study, the administration of gabapentin was found to substantially increase the ability the gastric mucosa to withstand damage caused by indomethacin or 96% ethanol. The mechanism by which gabapentin protects the gastric mucosa is not clear. The drug does not inhibit gastric acid secretion. Rather, an increase in gastric acid was observed in pyloric-ligated rats after systemic administration of gabapentin at doses of 50 or 100 mg/kg (Abdel-Salam and Sleem, 2009). Gabapentin was shown to exhibit antioxidative effects (Abdel-Salam et al., 2012), which may be of relevance to the effects observed herein.

5. CONCLUSION

This study provided evidence for the ability of gabapentin to maintain gastric mucosal integrity in face of the severe damage evoked by either indomethacin or ethanol.

Author contribution

OMEAS and NS conducted the research and analysis. OMEAS wrote and prepared the manuscript. OMEAS and NS approved the final version of the manuscript.

Informed consent

Not applicable.

Ethical approval

The experimental studies were done according to the recommendations of the Ethics Committee of the National Research Centre and the Guide for Care and Use of Laboratory Animals by the US National Institutes of Health (Publication No. 85-23, revised 1996).

Conflicts of interests

The authors declare that there are no conflicts of interests.

Funding

The study has not received any external funding.

Data and materials availability

All data associated with this study are present in the paper.

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