

Original Article	Effects of Indomethacin on The Gastric Mucosa of Adult Male Albino Rat and The Possible Gastroprotection by Alpha Lipoic Acid <i>Heba K. Mohammed and Hala Z. E. Mohamed</i> <i>Department of Human Anatomy and Embryology, Faculty of Medicine, Assiut University, Egypt</i>
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ABSTRACT

Background: Indomethacin is an effective NSAID used in inflammation treatment. However, it causes gastric mucosal lesions. Alpha lipoic acid (ALA) is an antioxidant that prevents cell damage.

Aim of the work: To detect the protective role of ALA on indomethacin-induced gastric mucosal alterations.

Material and Methods: A total number of thirty adult male albino rats were randomly equally divided into three groups: control group (received distilled water), indomethacin-treated group; received 50 mg/kg b.w of indomethacin., once orally and ALA + indomethacin - treated group; received 100 mg/kg b.w. of ALA, orally for 3 days followed by once administration of 50 mg/kg b.w. of indomethacin, orally 1h after the last dose of the ALA. Four hours after indomethacin administration. The rats were anesthetized, sacrificed and the stomach immediately removed and processed for histological (light and electron microscopic) and inducible nitric oxide synthase (iNOS) immunohistochemical examination. Area % of collagen fibers and iNOS of all animal groups was measured and compared.

Results: Indomethacin induced gastric mucosal erosion, degeneration, vacuolization, inflammatory cell infiltration, interstitial hemorrhage, blood vascular congestion and dilatation, deposition of collagen fibers and strong positive iNOS immunoreactivity. Area % of collagen fibers and iNOS was significantly increased in indomethacin-treated group when compared to control. ALA + indomethacin-treated group exhibited nearly normal histological, immunohistochemical and morphometric findings.

Conclusion: ALA has a gastroprotective role as it can prevent the deleterious effects of indomethacin on the gastric mucosa.

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Key Words: Alpha lipoic acid, gastric mucosa, indomethacin.

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INTRODUCTION

The stomach is important for food digestion. It resists noxious factors depending on a group of protective mechanisms^[1]. The stomach property depends on its mucosal barrier that permits it to contain an acid. Three protective components establish this barrier; a continuous epithelial cell lining, a surface mucus covering and the bicarbonate ions that act to neutralize harsh acids^[2, 3]. If this barrier is broken, the acid diffuses back into the mucosa causing stomach damage^[4].

The gastric mucosal lesions appear to be the result of the imbalances between the defensive factors and the destructive material levels^[5]. An oxidative stress, reduction of antioxidants, neutrophil accumulation, increased inflammatory

cytokines^[6] and reduced gastric mucosal blood supply have been accused in the development of stomach ulcers^[7, 8].

Non-steroidal anti-inflammatory drugs (NSAIDs) are popular as an anti-inflammatory treatment^[9]. Indomethacin is a synthetic NSAID with an analgesic and antipyretic activity. It is a potent inhibitor of the synthesis of prostaglandins which are important mediators of the inflammatory response^[10]. However, its usage in medicine is restricted because of its gastric injurious side effect^[4]. Indomethacin-induced gastric lesions were reported by many researches^[11-15]. An indomethacin-induced gastric ulcer was associated with an increased gastric acid secretion and a decreased mucosal nitrite level^[9].

Alpha lipoic acid (ALA) is an organo-sulfur compound derived from the octanoic acid. It is an antioxidant vitamin-like chemical^[16]. Yeast, liver, spinach, kidney, potatoes and broccoli are counted as good sources of the ALA. It is also made in the laboratory for usage in medicine as dietary supplements^[17]. The ALA is used for treatment of diabetes, memory loss and cardiovascular disorders^[18]. It also helps prevent cell damage and restore vitamin levels such as vitamins C and E^[19]. So, this study aimed to assess the possible role of ALA in protection of the gastric mucosa from indomethacin-induced insults in the albino rat.

MATERIAL AND METHODS

Chemicals:

□ Indomethacin (CAS ID: 53-86-1) and Alpha lipoic acid (CAS ID: 1077-28-7) were purchased from Sigma -Aldrich, St Louis Co., MO, USA.

□ Inducible nitric oxide synthase (iNOS) was purchased from Thermo Scientific Company, USA. Other reagents used in this study were high commercially grade.

Animals

A total number of thirty adult albino rats, weighing 200-220g, were obtained from the Animal Laboratory House of Assiut University. The animals were kept in the animal house in well-ventilated stainless steel cages at normal temperature ($22^{\circ}\text{C} \pm 5^{\circ}\text{C}$) under a 12:12 – hours light: dark cycle. They were fed with the standard diet. Water ad libitum was given to them throughout. The animals were dealt in accordance with the International Guidelines for the Care and Use of Laboratory Animals.

Experimental protocol

The rats were randomly classified into three equal groups. A control group; G1 (n=10, were given distilled water by oral route). Indomethacin-treated group; G2 (n=10, were fasted for food not water for twenty four hour, then, received a single oral dose of 50 mg indomethacin /kg b.w.^[20]. ALA+indomethacin-treated group; G3 (n=10), received 100 mg/kg b.w of ALA suspended in 100 ml distilled water^[16] orally once daily for 3 days and then, the rats received 50 mg indomethacin /kg b.w. one hour after the last dose of the ALA). Four

hours following indomethacin administration, rats of all animal groups were ether anesthetized and sacrificed. An intra-cardiac perfusion with saline was carried out and the abdomen was opened through a midline incision, then stomach was removed, opened along the greater curvature and gently washed with saline. Thereafter, some specimens from the stomach fundus of all animal groups were immersed in a neutral buffer formalin for light and iNOS immunohistochemical evaluations. Other specimens were immersed in 5% glutaraldehyde and processed for the scanning and transmission electron microscopic studies.

Histological study

Light microscopic study

The gastric fundus specimens were dehydrated in an alcohol of ascending grades. Then, they were embedded in paraffin. Sections of 5 μm -thick were taken, placed onto glass slides and deparaffinized. Some sections were stained with Haematoxylin and Eosin (H&E) and others with Masson's trichrome^[21] and examined by the light microscope.

Electron microscopic study

Scanning electron microscopy (SEM)

Specimens of the stomach fundus of all the animal groups were dehydrated by alcohols of graded series and liquid CO_2 . Stubs of aluminum were used to mount the specimens. Then, the specimens were fixed with a silver colloid and gold coated sputter according to Naguro and Breipohl^[22]. A jeol (J.S.M-5400 LV; Japanese Electron Optic Laboratory, Japan) in Assiut University, Electron Microscope Unit. was used to view and photograph the gastric fundus luminal surface.

Transmission electron microscopy (TEM)

Stomach fundus specimens of about 1x1mm of all the animal groups were rinsed in 0.1M cacodylate buffered glutaraldehyde (pH 7.4) for 24 h. Then, the specimens were dehydrated in graded ethanol, cleared using propylene oxide, post-fixed in osmium tetroxide placed in propylene oxide: Epon (1:1) overnight and embedded in Epon mixture pure. After that, the obtained blocks were put in oven at 60°C for three days. Semithin sections 0.5-1 μm in thickness were stained with Toluidine blue and examined by

a light microscope. On the other hand, ultrathin sections 450-500 Å in thickness were collected on copper grids and contrasted in uranyl acetate for 10 minutes and in lead citrate for 5 minutes^[23] and examined by a TEM (Jeol-JEM-100 CXII; Japanese electron optic laboratory, Tokyo, Japan) at the Electron Microscopic Unit of Assiut University.

Immunohistochemical study:

In order to evaluate the oxidative stress state, paraffin sections were immunohistochemically stained to detect the inducible nitric oxide synthase (iNOS). Therefore, streptavidin system with antibody against the iNOS marker was used. Sections were deparaffinized, hydrated and washed in phosphate-buffered saline (PBS). Then, they were treated with trypsin 0.01% for 10 minutes and washed with PBS for 5 minutes. The sections were incubated with a primary antibody (an overnight 1:100 diluted Rabbit Polyclonal Antibody at 4°C). Kits from Thermo Scientific Company (USA) were used. Detection of the immunoreactivity was done with 0.05% di-amino-benzidine and the slides were counterstained with Mayer's haematoxylin and then mounted. Consequently, an appearance of cytoplasmic brown coloration indicated positive iNOS immune reactivity^[24]. A normal rat serum (×100 diluted) was used instead of the primary antibody for negative controls.

Morphometric study:

Area % of collagen fibres (in Masson's trichrome-stained sections) and iNOS immune reactivity (in iNOS immunostained sections) of all animal groups were measured using Leica Qwin 500 Ltd image analysis computer system, Wetzlar, Germany. For carrying out these measurements, five non-overlapping high power fields in five sections which were randomly chosen in each group using an objective lens X400. All measurements were done per area of the view field.

Statistical Analysis

The morphometric results were introduced as mean ± standard deviation (SD). The data were evaluated with a Statistical Package for the Social Sciences (SPSS, 16.0). The statistical significance of differences was assessed by one way ANOVA,

followed by Dunnett's t-test. A *p*-value of ≤ 0.05 was considered statistically significant.

RESULTS

Gross appearance of the stomach mucosa:

The stomach mucosa of the control group (G1) showed a normal intact gastric mucosa (Fig. 1a). Indomethacin-treated group (G2) exhibited visible hemorrhagic mucosal lesions (Fig. 1b). The Indomethacin+ALA-treated group (G3) showed a nearly intact gastric mucosa (Fig. 1c).

I. Histological results

A. Light microscopic results

Haematoxylin and Eosin stain

The gastric fundic specimens of control animals was composed of mucosa, submucosa and gastric muscle layer. The normal mucosa was separated from the submucosa by a layer of smooth muscle called muscularis mucosa. Rows of columnar cells were forming the gastric pits. The mucosa was lined by mucous neck cells, parietal cells and chief cells. The parietal cells had a characteristic 'fried egg' appearance. They were spherical with a central nucleus, prominent nucleolus and an eosinophilic cytoplasm. Moreover, they were distributed through the length of the glands. The chief cells (zymogenic cells) were pyramidal with deeply-stained basal rounded nuclei. They were located between the parietal cells. The mucous cells were rounded with vesicular nuclei (Figs. 2a & 3a).

The gastric fundus specimens of indomethacin-treated animals showed a large haemorrhagic mucosal erosion, a sloughed gastric mucosal surface and a mucosal epithelial discontinuity. Also a disorganized glandular architecture, inflammatory cellular infiltration and degenerated, vacuolated and ruptured gastric mucosal cells were observed. Moreover, an interstitial haemorrhage, widening of the gastric glands with cellular debris inside the lumen, mitotic figures and homogenous acidophilic patches were seen (Figs. 2b & 3b, c, d).

The gastric fundus specimens of ALA+indomethacin-treated rats showed nearly normal gastric mucosa with intact glandular cells. The surface epithelial continuity was

re-established by intact surface mucous cells (Figs. 2c & 3e).

Masson's trichrome stain:

Sections of the control group revealed scanty amount of collagen fibres mainly found in the lamina propria, gastric muscle layer and submucosa (Fig. 4a). Indomethacin-treated group showed massive amount of collagen fibres deposited in the lamina propria, gastric muscle layer and submucosa (Fig. 4b). ALA+indomethacin-treated group revealed scanty amount of collagen fibres more or less similar to the control groups (Fig. 4c).

Toluidine blue stain:

Sections of the control group showed normal gastric columns covered with mucous layer. The gastric pits, lamina propria, mucous cells and parietal cells were observed (Fig. 5a). Indomethacin-treated group showed disrupted eroded areas, dilated and congested blood capillaries in the lamina propria, vacuolated cells, degenerated gastric cells and thick mucous layer on the eroded area (Figs. 5b, c, d). ALA+indomethacin-treated group exhibited nearly preserved mucosal architecture with more or less normal mucous and parietal cells. Less dilated blood capillaries were observed (Fig. 5e).

B. Electron microscopic results

SEM

Scanning electron microscope of the control fundic gastric mucosa viewed from the lumen revealed superficial cells with a cobblestone appearance. Some ropes of mucus extending from the gastric pits were observed (Figs. 6a, d). Mucosa of indomethacin-treated group was large eroded (Figs. 6b, e). ALA+indomethacin-treated group showed a preserved mucosa and ropes of mucus extending from the gastric pits (Figs. 6c, f).

TEM

Ultra-structurally, the parietal cells of the control group appeared large pyramidal with apical microvilli and contained intracellular canaliculi lined by numerous microvilli, electron

dense mitochondria, rounded nucleus and supranuclear Golgi apparatus (Fig. 7a). The chief cells of the control group showed basally located round nuclei with prominent nucleoli, mitochondria, packed cisternae of basal rough endoplasmic reticulum, free ribosome and Golgi apparatus. The cells were loaded with apical large translucent zymogenic secretory granules distributed throughout the cytoplasmic matrix (Fig. 8a). The enteroendocrine cells of the control group contained rounded nuclei, cytoplasmic small electron dense granules, poorly-developed rER and well-developed smooth endoplasmic reticulum (Fig. 9a).

As regards the ultrathin sections of the indomethacin-treated group, the parietal cells showed irregular nuclei with peripheral chromatin condensation, apoptotic nuclei with clumps of chromatin, dilated rER, dilated intracellular canaliculi, and intracellular canaliculi with disrupted microvilli, swollen amalgamated mitochondria and cytoplasmic vacuolization. Moreover, Golgi apparatus was poorly identified (Figs. 7b, c). The chief cells contained dilated rER, swollen disrupted mitochondria, secondary lysosomes, cytoplasmic vacuolation and hyperchromatic and irregular nuclei (Figs. 8b, c). Enteroendocrine cells contained disfigured nuclei with condensed peripheral chromatin, degenerated mitochondria and increased number of vacuolated secretory granules nearly filling the cytoplasm (Figs. 8b & 9b, c, d).

Apart from some vacuolated secretory granules seen in the enteroendocrine cells, the ultrastructure of the parietal (Fig. 7d), chief (Fig. 8d) and enteroendocrine (Fig. 9e) cells of the ALA+indomethacin-treated group showed nearly preserved cytoarchitecture to a great extent.

II. Immunohistochemical results

The gastric fundic mucosal cells of control group showed a weak immuno-reactivity to inducible nitric oxide synthase (iNOS) (Fig. 10a). In indomethacin-treated group, the fundic mucosal cells showed a strong positive immune reaction to iNOS which appeared as dark brown dots filling the cytoplasm (Fig. 10b). ALA+indomethacin-treated group revealed that the mucosal cells had a weak immune-positivity for iNOS (Fig. 10c).

III. Morphometric results

The mean area% of collagen fibers was significantly increased in indomethacin-treated group as compared to control and ALA+indomethacin-treated groups. The mean area % of collagen fibers of ALA+indomethacin-treated group showed non-significant change compared to control group ($p>0.5$) (Table 1 & Histogram 1).

Regarding the area% of iNOS, there was a significant increase in indomethacin-treated group as compared to control and ALA+indomethacin-treated groups. Area% of iNOS of ALA+indomethacin-treated group showed non-significant change compared to control group ($p>0.5$) (Table 2 & Histogram 2).

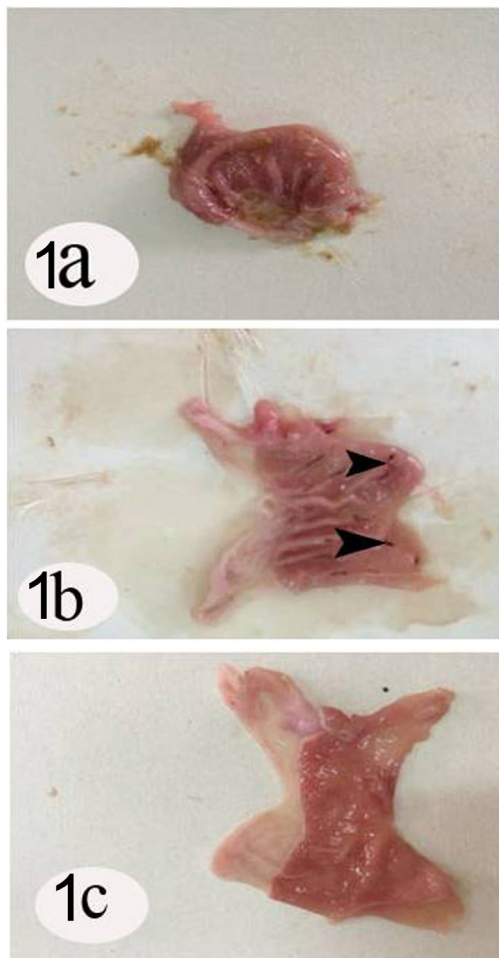


Fig 1: Photographs of the stomach mucosa. (1a) Control group showing normal intact gastric. Mucosa; (1b) Indomethacin-treated group showing visible hemorrhagic mucosal lesions (arrow heads); (1c) Indomethacin+ALA-treated group showing intact surface.

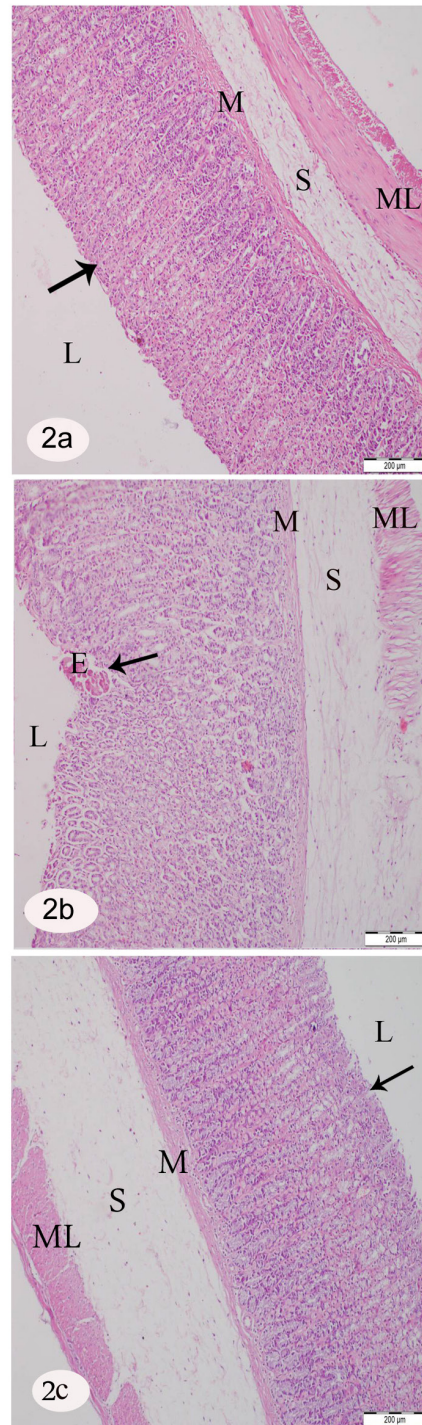


Fig. 2: Photomicrographs of paraffin sections stained by H&E in the gastric fundus. (2a) Control group showing normal intact surface epithelium (arrow). Note the muscularis mucosae (M), submucosa (S) and gastric muscle layer (ML); The lumen of the stomach (L) is noticed. (2b) Indomethacin-treated group showing eroded surface epithelium (arrow) with haemorrhage (E) and disorganization of the adjacent glandular architecture; (2c) Indomethacin+ALA-treated group showing nearly intact surface epithelium (arrow). H&E, X100.

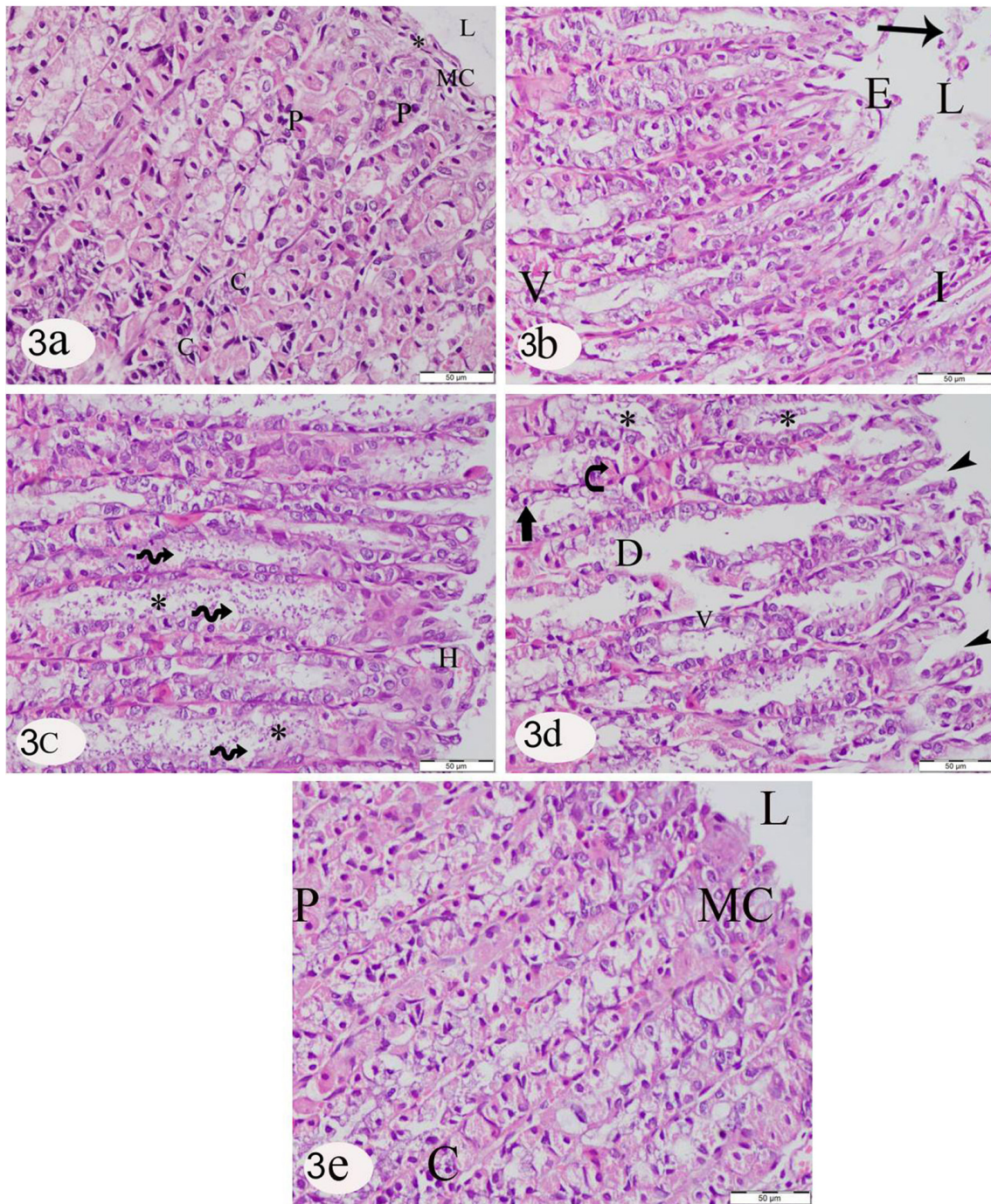


Fig. 3: Photomicrographs of paraffin sections stained by H&E in the gastric fundus. (3a) Control group showing normal parietal cells (P), chief cells (C) and mucous cells (MC). Notice the lumen of the stomach (L) and gastric pits (asterisks); (3b-d) Indomethacin-treated group showing a large erosion with a mucosal epithelial discontinuity (E), a sloughed off surface gastric mucosa (arrow) in the gastric lumen (L), inflammatory cellular infiltration (I) and vacuolated cells (V). Note the interstitial haemorrhage (H), widening of the gastric glands with a cellular debris inside the lumen (asterisks), mitotic figures (thick arrow), rupture of glandular cells (wavy arrows) and minute surface erosions (arrow heads). Homogenous acidophilic patches (curved arrow) and degeneration of gastric cells (D) can be noticed; (3e) Indomethacin+ALA-treated group showing nearly normal gastric mucosa, preserved gastric gland cells, intact parietal cells (P), chief cells (C) and surface mucous cells (MC). Gastric lumen (L) can be seen. H&E, X400.

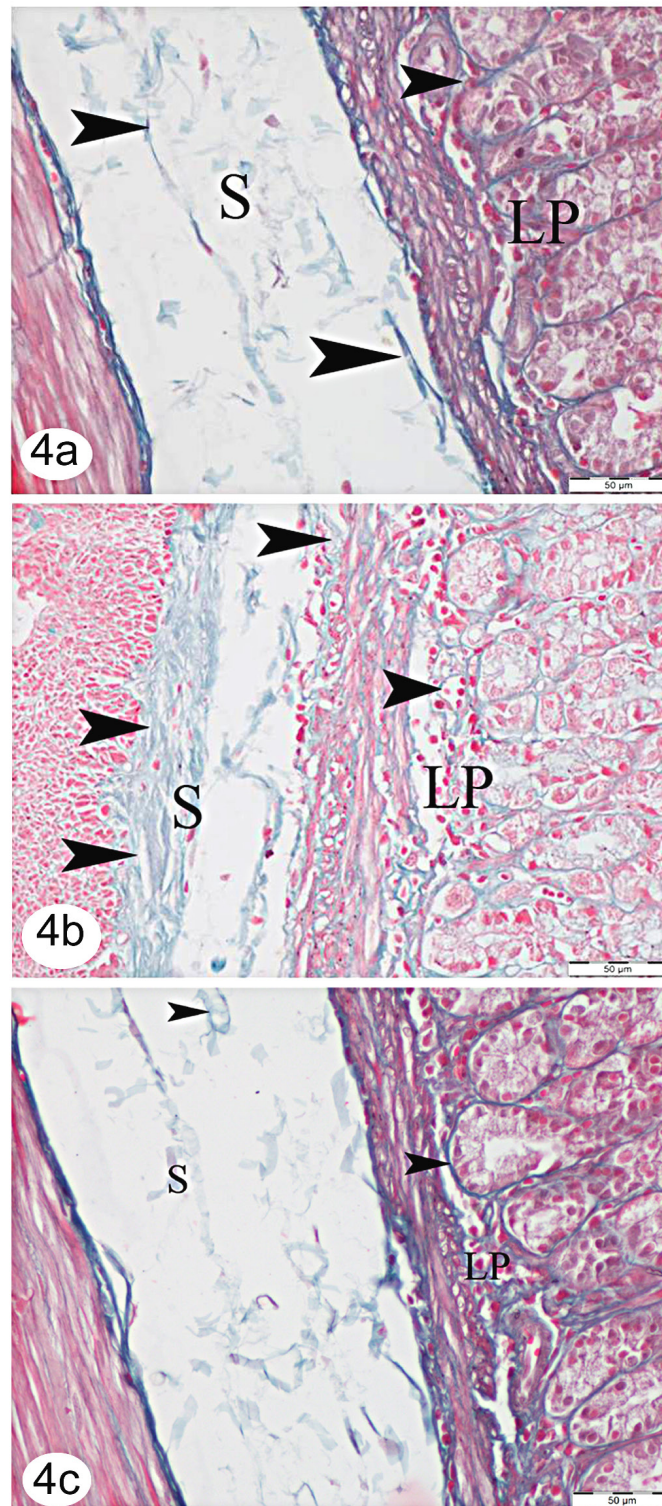


Fig. 4: Photomicrographs of the gastric fundus of the adult albino rat . (4a) control group showing scanty amount of collagen fibres (arrow heads), observed mainly in the lamina propria (LP) and submucosa (S); (4b) indomethacin-treated group showing massive amount of collagen fibres (arrow heads) deposited in the lamina propria (LP) and submucosa (S); (4c) Indomethacin+ALA-treated group showing remarkable reduction of the amount of collagen fibres (arrow heads) in the lamina propria (LP) and submucosa (S) as compared with that in indomethacin treated group. Masson's trichrome; X400.

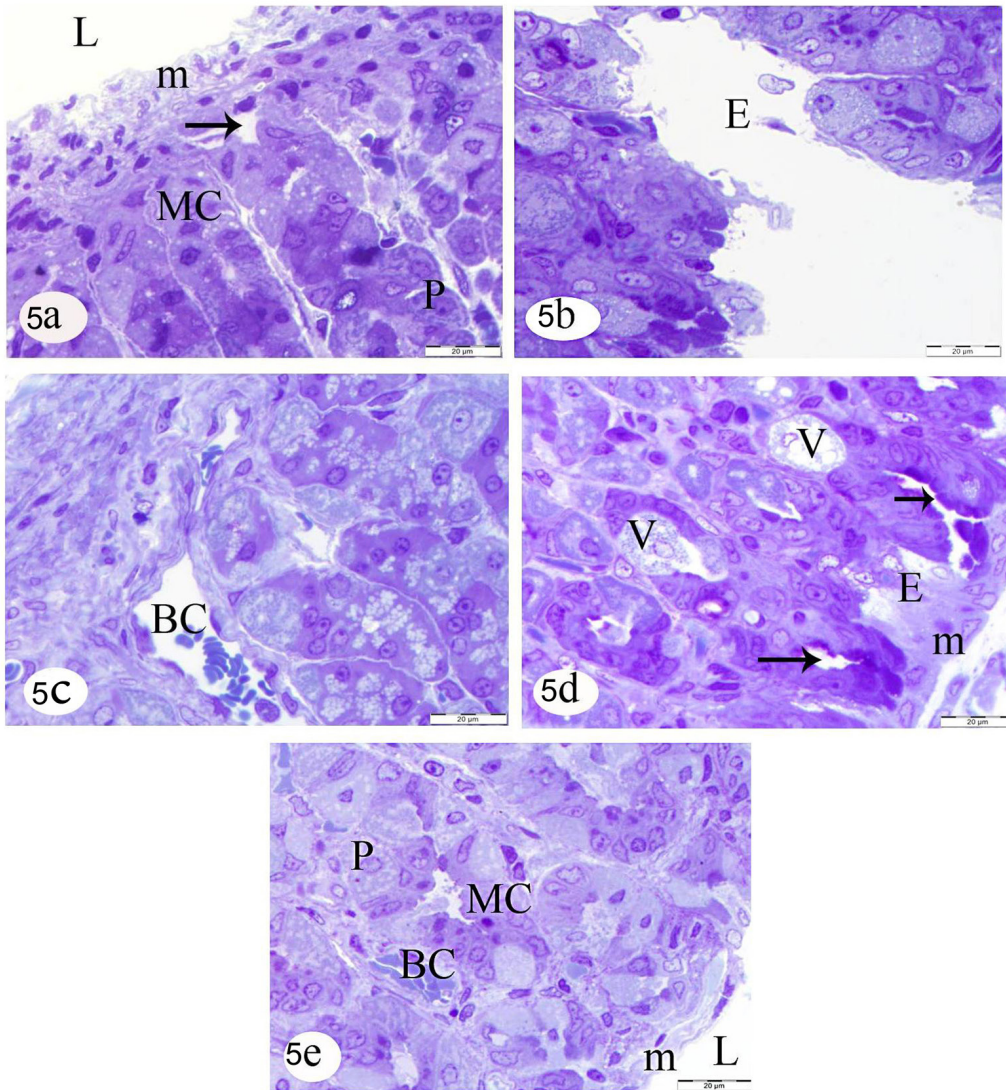


Fig. 5: Photomicrographs of the rat gastric fundus. (5a) control group showing normal gastric columns covered with mucous layer (m). Notice the gastric pits (thin arrow), lumen of stomach (L), mucous cells (MC) and parietal cells (P); (5b-d) Indomethacin-treated group showing erosion of gastric mucosa (E), congested and dilated blood capillaries (BC) in the lamina propria, vacuolated cells (V). Notice the thick mucous layer (m) on the eroded area (E) and the gastric pits (arrows); (5e) Indomethacin+ALA-treated group showing nearly normal mucous (m), mucous cells (MC) and parietal cells (P). Less dilated blood capillaries (BC) and stomach lumen (L) can be noticed. Toluidine blue, X1000.

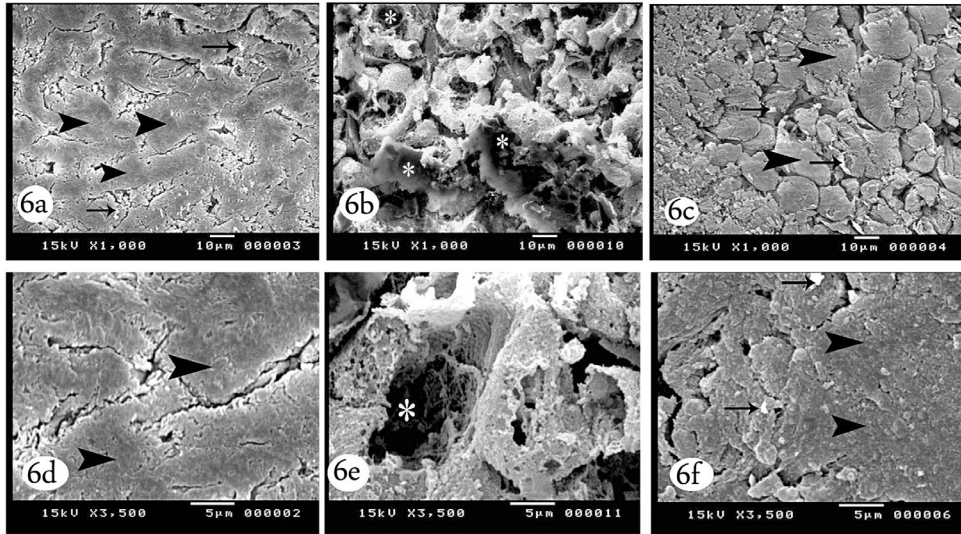


Fig. 6: SEM of the rat gastric fundic mucosa, viewed from the luminal aspect. (6a,d) Control mucosa showing, superficial cells exhibiting a cobblestone appearance (arrow heads). Note some ropes of mucus extending from gastric pits (arrows). SEM X1000, X3500, respectively); (6b,e) Mucosa of indomethacin-treated group showing erosions (asterisks). SEM X1000, X3500, respectively); (6c,f) Mucosa of indomethacin+AIA-treated group showing healing mucosa (arrow heads) and ropes of mucus extending from the gastric pits (arrows). SEM, X1000, X3500, respectively.

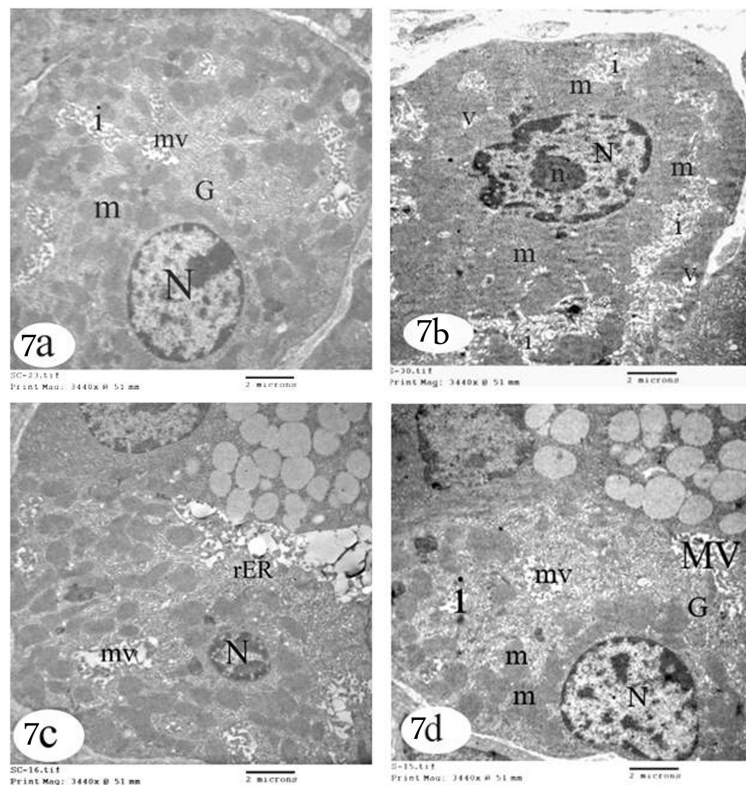


Fig. 7: TEM of the parietal cells of rats' gastric glands showing (7a) the control group, the cell appear large, pyramidal with a rounded nucleus (N), intracellular canaliculi (i) lined by numerous microvilli (mv), electron dense mitochondria (m) and supranuclear Golgi apparatus (G); (7b) Indomethacin-treated group, contains an irregular nucleus (N) with a peripheral chromatin condensation and prominent large nucleolus (n), apparently dilated intracellular canaliculi (i), cytoplasmic vacuolization (v) and swollen and amalgamated mitochondria (m). Notice that Golgi apparatus is poorly identified; (7c) Indomethacin-treated group, contains an apoptotic nucleus with clumps of chromatin (N), dilated rough ER (rER) and disrupted microvilli in the intracellular canaliculi (mv); (7d) Indomethacin+AIA-treated parietal cell containing rounded nucleus (N), electron dense mitochondria (m), intracellular canaliculi (i) and supranuclear Golgi apparatus (G). Notice the apical microvilli (MV). TEM, X5800.

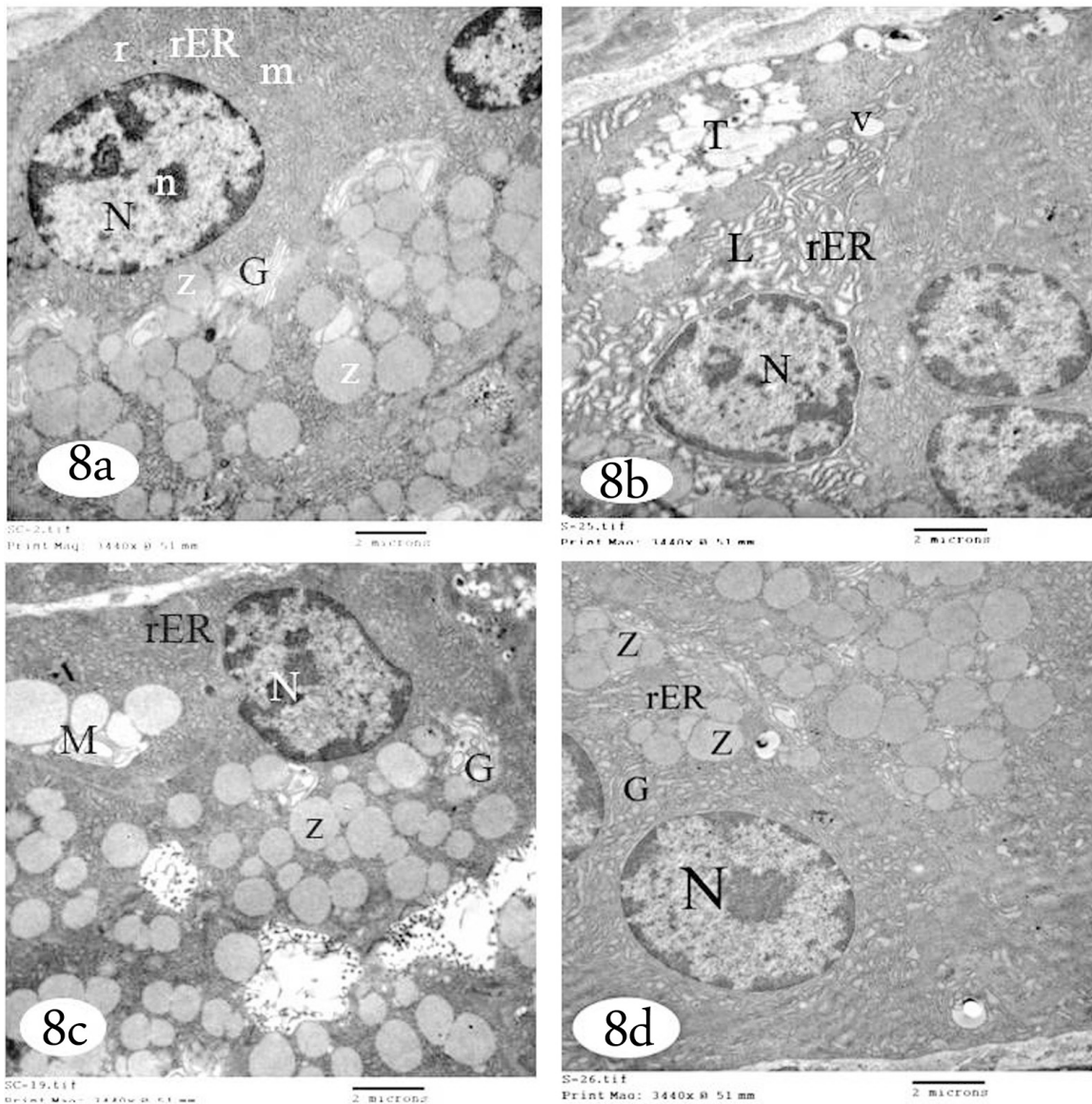


Fig. 8: TEM of the chief cells of rats' gastric glands showing (8a) control group chief cell, containing basally located round nucleus (N) with prominent nucleolus (n), mitochondria (m), packed cisternae of rough ER (rER), free ribosomes (r), Golgi apparatus (G) and apical large translucent zymogenic secretory granules (Z) distributed throughout the cytoplasmic matrix; (8b) Indomethacin-treated chief cell containing an irregular nucleus (N), a dilated rough ER (rER), cytoplasmic vacuolization (V), secondary lysosomes (L). Notice a neighboring enteroendocrine cell (T) with vacuolated secretory granules; (8c) Indomethacin-treated chief cell containing a hyperchromatic irregular nucleus (N), a dilated rough ER (rER), a myelin figure (M) and a dilated perinuclear Golgi apparatus (G). Notice zymogenic secretory granules (Z); (8d) Indomethacin+A1A-treated chief cell containing a round nucleus (N), a Golgi apparatus (G), a rough ER (rER) and zymogenic secretory granules (Z).
TEM, X5800.

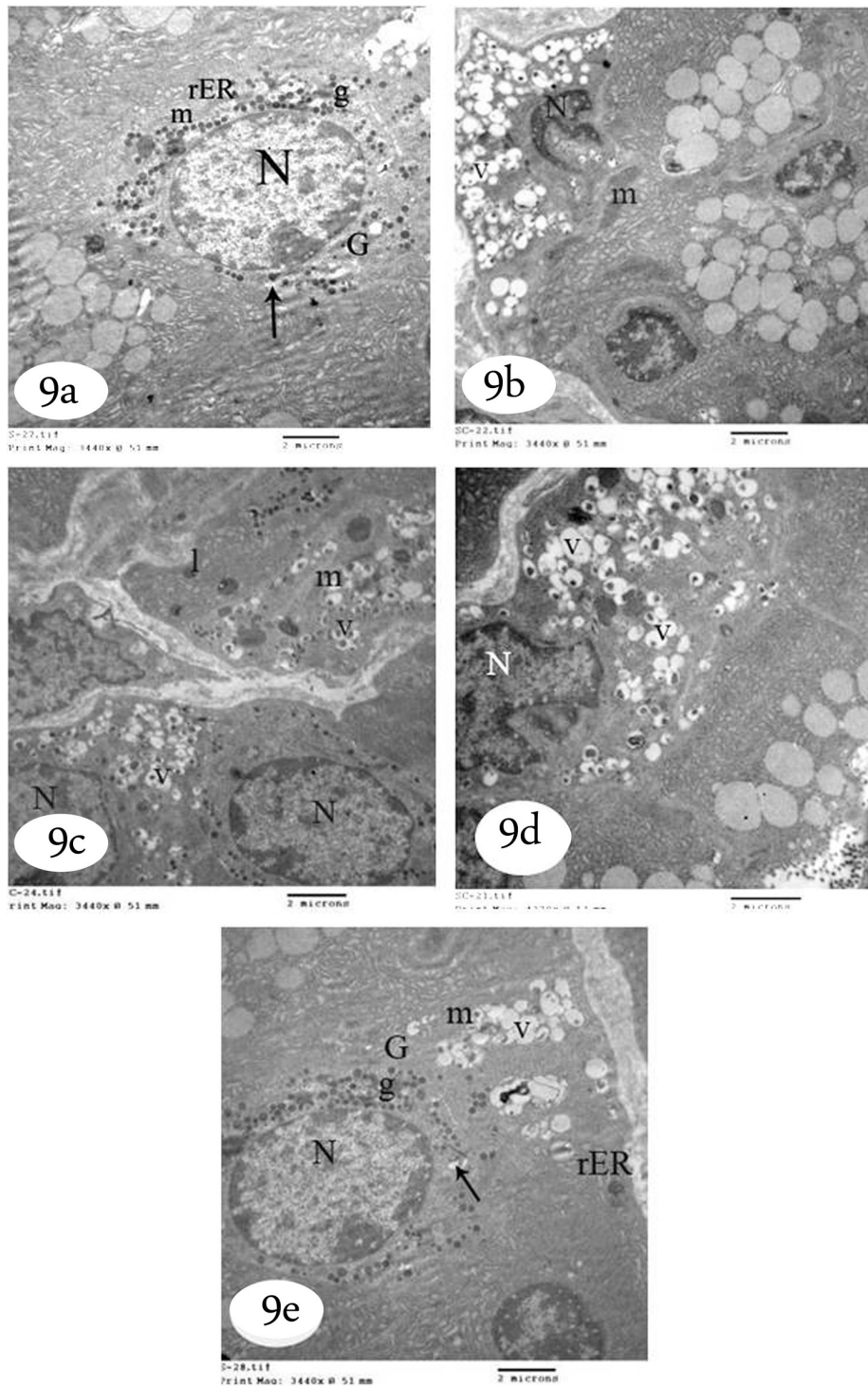


Fig. 9: TEM of the enteroendocrine cells of rats' gastric glands (9a) the control enteroendocrine cell characterized by cytoplasmic electron dense granules (g), a poorly-developed rough ER (rER) and a well-developed smooth endoplasmic reticulum (arrow), a Golgi apparatus (G), mitochondria (m) and a rounded nucleus (N) (TEM, X5800); (9b-d) Indomethacin-treated enteroendocrine cell containing irregular indented shrunken nuclei (N) with condensed peripheral chromatin and apparently increased amount of vacuolated secretory granules (v) nearly filling the cytoplasm, degenerated electron dense mitochondria (m) and lysosomes (l) (TEM, X5800, X5800, X7200, respectively); (9e) Indomethacin+ALA-treated enteroendocrine cell restoring the normal cytoarchitecture. Notice the nucleus (N), secretory granules (g), rough ER (rER), smooth endoplasmic reticulum (arrow), Golgi apparatus (G) and mitochondria (m). Some vacuolated secretory granules are seen (v). TEM, X5800.

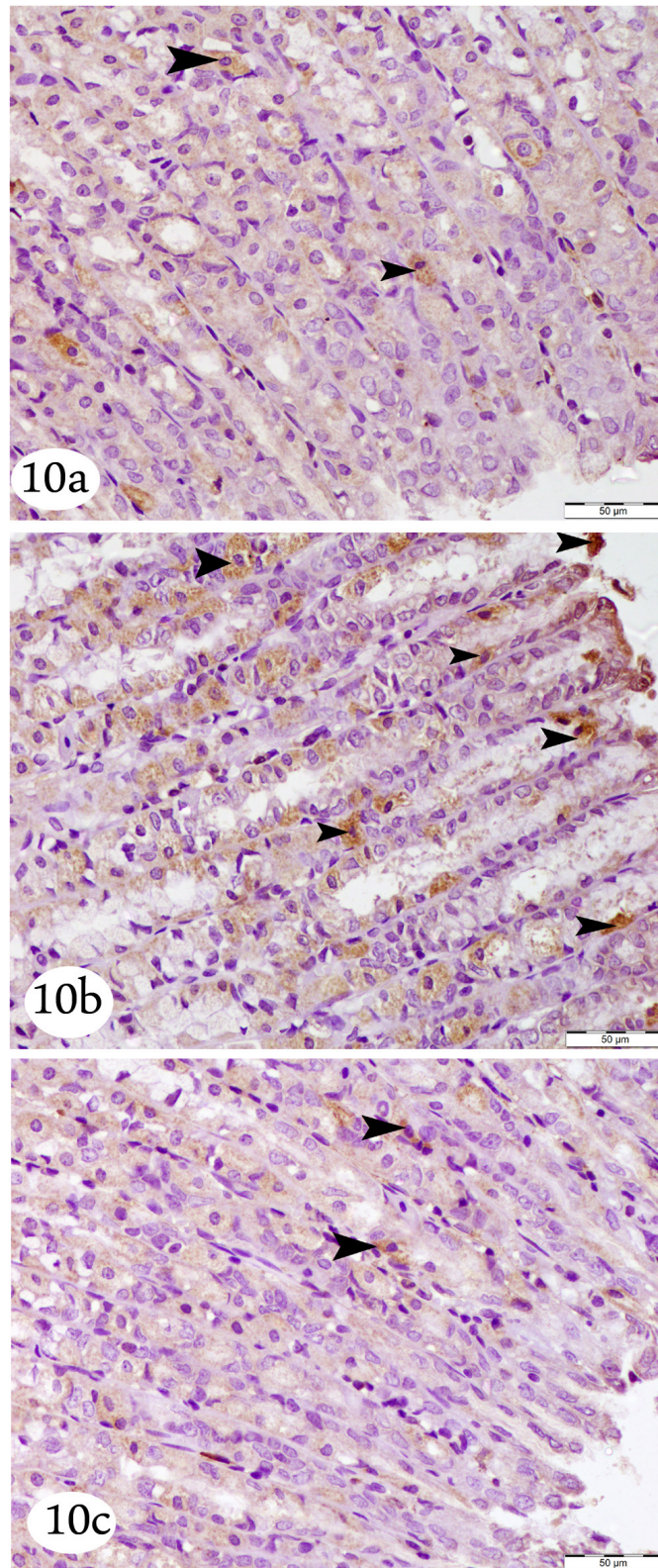
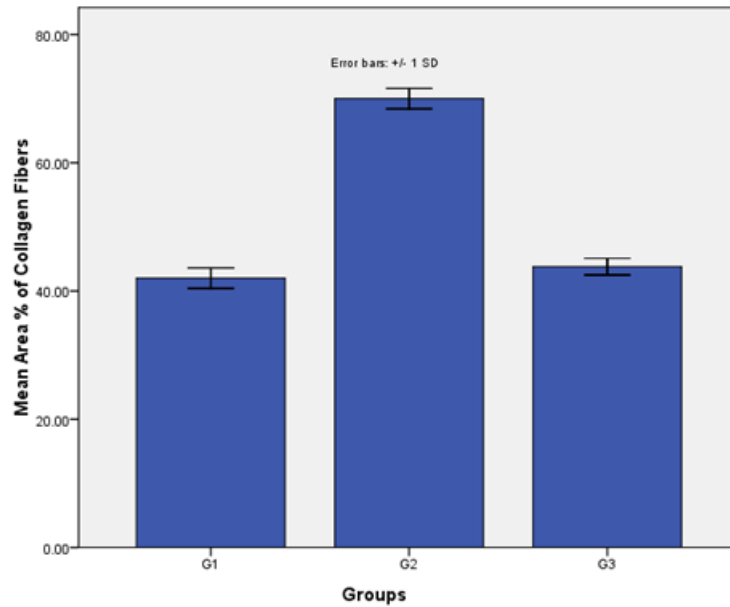


Fig. 10: iNOS immune-reactivity photomicrographs of the rats' gastric fundic mucosa. (10a) control group showing weak immune reaction to iNOS (arrow heads) (x400); (10b) Indomethacin-treated group showing strong positive immune reaction to iNOS (arrow heads) (x400); (10c) Indomethacin+AIA-treated group showing weak immune-positivity for iNOS (arrow heads). iNOS immunostain, X400.

Table 1: Mean area % of collagen fibres.

	G1	G2	G3	<i>p-value</i>	
				G1vs G2	G1vs G3
Mean ± SD	42.01±1.58	70.00±1.57	43.80±1.34	0.000*	0.081

*Differences at $p < 0.05$ were considered significant.

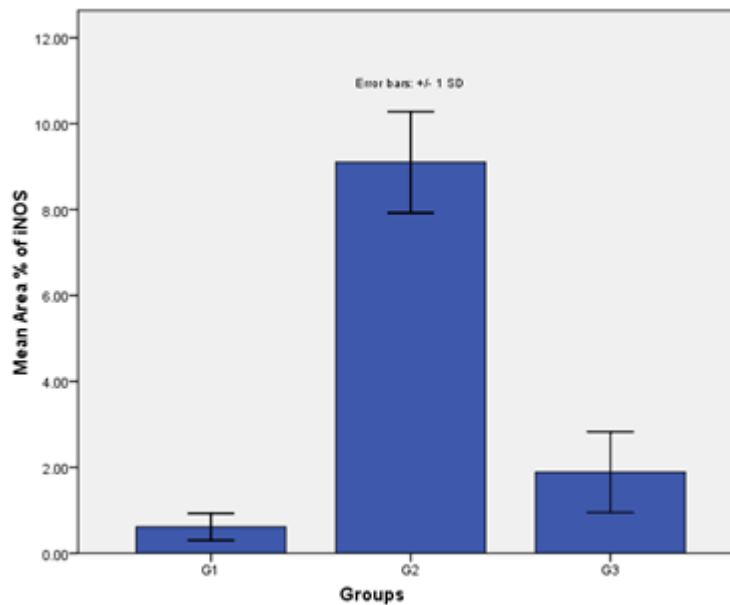


Histogram 1: Mean area % of collagen fibres.

Table 2: Mean area % of iNOS.

	G1	G2	G3	<i>p-value</i>	
				G1vs G2	G1vs G3
Mean ± SD	0.65±0.19	9.10±1.17	1.63±0.62	0.000*	0.066

*Differences at $p < 0.05$ were considered significant.



Histogram 2: Mean area % of iNOS.

DISCUSSION

The stomach ulcer is a common gastrointestinal disorder^[25], which occurs due to the imbalances between the offensive and defensive factors^[26,31]. It is an illness that affects a considerable number of people worldwide^[27,51].

At the same time, a growing body of evidence demonstrated that the NSAIDs are widely prescribed drugs because they are useful as analgesic and anti-inflammatory agents^[28,29]. Wallace^[30] stated that indomethacin, is widely used in the treatment of osteoarthritis, rheumatoid arthritis and chronic pain. However, it is involved as an established cause of stomach ulcer in humans and rats^[31]. ALA is an important natural antioxidant^[32]. So, this study was established in order to assess the antiulcer effects of the ALA in indomethacin-induced stomach ulcer in the male albino rats.

The gross and histological pictures of the stomach sections of the present work demonstrated that indomethacin induced hemorrhagic erosions, mucosal epithelial discontinuity, vacuolated cells, sloughed off surface gastric mucosa, degeneration of the gastric cells and widening of the gastric glands with cellular debris inside the lumen. These results were similar to the findings of Shalaby *et al.*^[33] who observed a sloughed epithelium, superficially located ulcers and severe necrosis, due to indomethacin treatment. Wallace^[34] reported that, the NSAIDs induced gastric ulcers and hemorrhage. They indorsed that an inactivation of several growth factors which are important in the mucosal defense and repair was detected.

Hayder and Al-Shawi^[35] and Harada *et al.*^[36] reported that the NSAIDs caused a cellular damage in the stomach, intestine and colon through autophagy and apoptotic processes induced in the intestinal epithelial cells. Moreover, Maity *et al.*^[37] indicated that indomethacin activated the caspase-3-dependent pathway for gastric mucosal injury.

Yokoe *et al.*^[38] added that the NSAIDs-induced epithelial cell damage occurred not only in the stomach but also in the intestines and colon and the protein degradation of collagen I and HIF-1 α might play an important role. A study by Murat *et al.*^[39] has reported that, the indomethacin

generated reactive oxygen species (ROS) that played an important role in the stomach ulcer formation.

In the present work, there was an inflammatory cellular infiltration of the fundic gastric lamina propria of the indomethacin-treated group. This comes in agreement with Shalaby *et al.*^[33] who observed a heavy cellular infiltration in the ulcer beds of the fundic lamina propria. They reported that, the NSAIDs affected the intercellular junctional integrity and inhibited the protective mucus layer production. Accordingly, the mucosa became exposed to the effect of the acid and proteolytic enzymes then invaded by the bacteria. The same finding was also observed by de Carvalho *et al.*^[40] who observed gastric mucosal inflammatory cellular infiltration in patients of gastritis and gastric ulcers. Moreover, Palacios-Espinosa *et al.*^[41] added that, polymorphonuclear leucocyte infiltration may be due to an infection with *Helicobacter pylori* which is related to NSAIDs ulcers. Kangwan *et al.*^[42] stated that neutrophils are involved in the inflammation and injury of a variety of tissues such as the gastric mucosa. An activation of neutrophils released superoxide anion, hypochlorous acid and proteases. Saxena and Singh^[43] added that neutrophils can also migrate into the surrounding tissues resulting in further disruption of the stomach mucosa.

Elahi *et al.*^[44] and Yildirim *et al.*^[45] attributed the anti-inflammatory effect of indomethacin to the suppression of vasodilator prostaglandin E2 and prostaglandin 1, 2 synthesized from arachidonic acid through cyclo-oxygenase pathway. Indomethacin-induced stomach ulcers occur via this pathway and the inhibition of constitutive NO synthase^[46]. NO production from iNOS interacts with ROS to form peroxynitrite, which is highly cytotoxic and damages epithelial cells. The acidic nature of NSAIDs can also kill epithelial cells as mentioned by Tiong *et al.*^[31].

Taiwo and Conteh^[47], Qazi *et al.*^[48] and Ribeiro *et al.*^[29] stated that gastric lesions caused by indomethacin were mainly due to the reduction of prostaglandin synthesis. It is well known that prostaglandins stimulate mucus and bicarbonate secretion as well as inhibit acid secretion^[30]. Thus, the decreased prostaglandin level impairs the stomach mucosal protection and increases acid secretion producing ulcer^[49].

The present study revealed excessive amount of collagen fibres deposited in the lamina propria and submucosa of the indomethacin-treated group. Hagraş *et al.*^[50] observed the same finding after treatment with the anti-rheumatic drug Leflunomide. They attributed that to the closely interrelation between the cellular infiltration and fibrosis as reported by Pochetuhēn *et al.*^[51] who explained clearly the associated increased deposition of collagen fibres by the activated fibroblasts which were depicted hand in hand with the cellular infiltration. They added that the releasing of TNF- alpha through activated macrophages stimulates the release of many chemokines from the macrophages, epithelial cells and fibroblasts.

The present study revealed that indomethacin-treated group led to disrupted eroded areas, dilated and congested blood capillaries in the lamina propria, vacuolated cells, degenerated gastric cells and thick mucous layer on the ulcerated area. The vascular affection in indomethacin-treated rats' gastric mucosa in the form of congestion and dilatation of the blood vessels comes with the observation of Morini *et al.*^[52] who stated that microvascular damage was the earliest event following aspirin and indomethacin, preceding leading to epithelial lesions. Moreover, Saeidnia and Abdollahi^[53] reported that the NSAIDs primarily target the microvascular endothelium in the stomach causing its perforation. The findings of dilated gastric glands and thick mucous were in harmony with Hagraş *et al.*^[50] who attributed the excessive production of mucous secretion by mucous neck cells as an attempt by the body to safe guard against more damage of the gastric surface coat, or, it might be related to inhibition of prostaglandin I₂ production, which is considered as a potent anti-secretory agent as stated by Ng *et al.*^[54].

The electron microscopic study of the gastric sections of the indomethacin-treated group of the present work revealed that the parietal cells had irregular nuclei with peripheral chromatin condensation, dilated intracellular canaliculi, cytoplasmic vacuolations, Golgi apparatus was poorly identified and amalgamated mitochondria. The chief cells contained dilated rER, swollen disrupted mitochondria, secondary lysosomes, cytoplasmic vacuolation and hyperchromatic and irregular nuclei. The enteroendocrine cells contained irregular indented shrunken nuclei

with condensed peripheral chromatin, lysosomes, degenerated mitochondria and increased number of vacuolated secretory granules nearly filling the cytoplasm.

These results are in agreement with Hagraş *et al.*^[50] and Shalaby *et al.*^[33] who confirmed that the early signs of parietal cell damage were disruption of their canaliculi and presence of dense bodies which were secondary lysosomes. Shalaby *et al.*^[33] reported that the intracellular canaliculi disrupted and dilatated leading to parietal cells vacuolizations. Polat *et al.*^[55] suggested that, an impairment of the tight junctional complex and permeability between the gastric mucosal epithelial cells may be implicated in NSAIDs-induced chief cells disorder.

The affection in the enteroendocrine cells in indomethacin-treated group of the present study comes with the observation of Stachura *et al.*^[56] who found argyrophilic cells, singly or in groups, in the lamina propria of surgically obtained stomach specimens of patients of gastric ulcer. They concluded that the argyrophilic reaction led to secretion of gastrin, which acted on the fundic glands and strongly promoted hydrochloric acid secretion. Morris and Wallace^[57] reported that the acid promotes hemorrhage through platelet thrombi destruction, breaking down the fibrin network and removing the substratum necessary for orderly epithelial re-establishment and by providing an unstirred layer for the neutralization of hydrogen ions by gastric bicarbonate secretion.

The present results regarding the stomach mucosal morphology were confirmed by the immunohistochemical findings where the sections of indomethacin-treated group showed an intense positive reaction to iNOS which appeared as dark brown dots filling the cytoplasm. These results also coincide with Wang *et al.*^[58] who detected similar findings in aspirin-treated rats' stomach. Cherdantseva *et al.*^[59] reported that the cellular inflammatory infiltrates composed of lymphocytes, macrophages and plasma cells. They added that they secreted active substances like iNOS.

Regarding the area % of collagen and iNOS in gastric fundic mucosa, the present work exhibited a significant increase in indomethacin-treated group's values as compared to those of control and indomethacin+AIA-treated groups. These results

were in parallel with the present histological and immunohistochemical ones.

The present study demonstrated the protective effect of the ALA administration on the histological, immunohistochemical and morphometrical findings. The current result runs parallel with the reports documented by Kaplan *et al.*^[60] who observed that the ALA protects against indomethacin-induced gastric oxidative toxicity by modulating the antioxidant system by interfering with the prooxidant effects of NSAIDs and binding free radicals directly or indirectly and enhancing the effectiveness of other antioxidants.

Sehirli *et al.*^[16] and Hussein *et al.*^[17] studied the simultaneous treatment with the ALA and ethanol in rats and found that the ALA increased the antioxidant enzymes, sialic acid and vitamin C. On the other hand it reduced the DNA fragmentation and lipid peroxidation. They suggested that, ALA seems to help prevent the cellular damage, restores vitamin levels and thus it might be effective in counteracting and healing enhancement of the deleterious effect of the gastric ulcers by its radical scavenging and antiapoptotic activity, as well as by regenerating the endogenous antioxidant mechanisms. Hence, it can decrease the effect of (ROS) and nitrogen species that can cause oxidative damage to gastric mucosa as mentioned also by Shay *et al.*^[19].

Moreover, Abd El-Kader *et al.*^[4] observed that ALA recovered rats' models of gastric ulcers and attributed this finding to role of ALA in reducing gastric secretion volume and increasing pH value and mucous secretion. Karakoyun *et al.*^[32] reported that ALA treatment had a role in the healing process of acetic acid-induced gastric injury in rats via the suppression of neutrophil accumulation, preservation of endogenous glutathione, inhibition of apoptosis and ROS generation.

CONCLUSIONS

Indomethacin has an injurious impact on the stomach mucosal lining that could be prevented by the administration of ALA. Thus, ALA has gastroprotective effects.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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التأثيرات الناتجة عن تناول عقار إندوميثاسين على الغشاء المخاطي للمعدة في الفأر الذكر البالغ والدور الوقائي المحتمل لحمض ألفا ليبويك

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ملخص البحث

المقدمة: يعتبر إندوميثاسين عقار فعال في علاج الالتهاب. ومع ذلك، فإنه يسبب إصابة الغشاء المخاطي المعدي. حمض ألفا ليبويك ((ALA) مضاد للأكسدة ويمنع تلف الخلايا.

الهدف من البحث: لتقييم الدور الوقائي المحتمل لحمض ألفا ليبويك في علاج التغيرات التي يسببها تناول عقار الإندوميثاسين على المعدة.

المواد وطرق البحث: تم تقسيم ثلاثين من ذكور الجرذان البالغة عشوائياً بالتساوي إلى ثلاث مجموعات: المجموعة الضابطة (أعطيت الماء المقطر)، المجموعة المعالجة بالإندوميثاسين (أعطيت ٥٠ ملجم إندوميثاسين / كجم من وزن الجسم، مرة واحدة عن طريق الفم) و المجموعة المعالجة بكل من ALA + إندوميثاسين (تلقت ١٠٠ ملجم ALA / كجم من وزن الجسم، مرة واحدة عن طريق الفم لمدة ٣ أيام ثم بعد ساعة من تناول آخر جرعة من حمض ألفا ليبويك أعطيت ٥٠ ملجم إندوميثاسين / كجم من وزن الجسم، مرة واحدة عن طريق الفم). بعد أربع ساعات من تناول عقار إندوميثاسين، تم تخدير الفئران ثم التضحية بها، وأزيلت معدتهم على الفور، وتمت معاملتها للدراسة الهستولوجية (عن طريق الفحص بالمجهرين الضوئي والإلكتروني) والدراسة الهستوكيميائية مناعية باستخدام دليل على وجود الانزيم المستحث المخلق لأوكسيد النيتريك (iNOS). تم قياس النسبة المئوية لكل من مساحة ألياف الكولاجين و iNOS لجميع المجموعات ومقارنتها.

النتائج: لقد سبب تناول عقار إندوميثاسين تغيرات في الغشاء المخاطي للمعدة في شكل تاكل ، تحلل، فجوات ، تسلل خلايا التهابات، نزف في الفراغات بين الخلايا، ترسب كمية كبيرة من ألياف الكولاجين واتساع واحتقان في الأوعية الدموية وتفاعل إيجابي ملحوظ للإنزيم المستحث المخلق لأوكسيد النيتريك (iNOS). و لقد لوحظ زيادة معنوية في نسبة مساحة ألياف الكولاجين و iNOS في المجموعة المعالجة بالإندوميثاسين بالمقارنة بالمجموعة الضابطة. ولقد أظهرت المجموعة المعالجة بكل من ALA + إندوميثاسين ان النتائج الهستولوجية والهستوكيميائية مناعية والإحصائية قريبة من الطبيعي.

الاستنتاج: أن ALA يقوم بدور وقائي لحماية المعدة حيث أنه يستطيع منع حدوث التأثيرات الضارة لعقار إندوميثاسين على الغشاء المخاطي للمعدة.