Comparative Histological and immunohistochemical study on the effect of curcumin and wild honey versus omeprazole on a rat model of gastric ulcer

Original Article

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ABSTRACT

Background and objectives: Gastric ulcers are among the most common diseases affecting humans. Omeprazole, a proton pump inhibitor, is widely used in the treatment of peptic ulcer. Wild honey alone, or in combination with other natural products, has been used to treat gastric ulcer. This study aimed to investigate the possible curative role of curcumin combined with wild honey in comparison to omeprazole on aspirin- induced gastric mucosal damage in adult male albino rats.

Methods and Results: Thirty adult male albino rats were divided into: Group1 (control group), group2 (ulcer group) (received 200mgkg BW aspirin orally). On day 6, rats of group2 were equally subdivided into; subgroup 2a (continued Aspirin), subgroup 2b (received combined curcumin and honey) and subgroup 2c (received 20mgkg BW omeprazole orally). All rats were sacrificed at day 11. Fundic specimens were processed for H &E, PAS, and immunohistochemical stain for COX-2, followed by morphometric assessment and statistical analysis. Aspirin-induced ulcer was evidenced by sloughing of surface epithelium, widened fundic glands, vacuolated surface and mucous neck cells and some vacuolated or shrunken oxyntic with pyknotic nuclei, in addition to significant increase in COX-2 immunoexpression. Combined curcumin and honey improved these histological alterations with a significant decrease in COX-2 immunoreactivity.

Conclusion: the combination of curcumin and wild honey healed most of the deleterious morphological changes of aspirin-induced gastric ulcer, with a comparable effect to omeprazole. This offers an alternative treatment of peptic ulcer to avoid adverse drug reactions.

INTRODUCTION

Peptic ulcer is a worldwide chronic disease which impairs the quality of life, associated with increased morbidity and mortality[1]. In 80% of the cases, gastric ulcer is caused primarily by the use of nonsteroidal anti-inflammatory drugs, 10% by Helicobacter pylori and about 8-10% by eating spicy and fast food[2].

Aspirin is a potent nonsteroidal anti-inflammatory drug (NSAID) that is used for the treatment of rheumatoid arthritis and related diseases as well as the prevention of cardiovascular thrombotic diseases. Gastric ulcer associated with the use of aspirin is a major problem[3].

There are two main approaches for treatment of peptic ulcer. Reduction of gastric acid production and enhancing gastric mucosa protection[4].

Curcumin, a commonly used spice and food-coloring agent, has been used in traditional remedy for a wide range of ailments, including wound healing, urinary and gastrointestinal tract infections[5]. It has considerable gastro-protective and anti-ulcerogenic effects[2].

Honey has been long known for its preventive and curative effect in medicine. It also exhibits anti-tumor activity, with pronounced anti-metastatic and anti-angiogenic effects in addition to its anti-inflammatory, immune-stimulant, antiulcer and wound/burn-healing properties[6]. It is believed that by consuming the combination of honey and herbs, the therapeutic outcome could be more effective than by using it alone[7].

Proton pump inhibitors (PPIs) are widely prescribed as first-line treatment in the prevention and therapy of peptic ulcer[8]. Omeprazole, a proton pump inhibitor (PPI), is one of the most potent gastric acid reducers[9]. Its adverse effects include headache, diarrhea, abdominal pain, nausea, dizziness, sleep deprivation[10] and has also been associated with osteoporosis related fractures[11].

Therefore, new treatments have been sought to enhance the efficacy of current drugs or to discover potential new agents that are more effective, less expensive and have fewer health-associated side effects than those currently used[12].

Accordingly, this work was designed to evaluate the possible curative role of a combination of curcumin and wild honey in comparison to omeprazole on aspirin- induced gastric mucosal damage in adult male albino rats. This effect was assessed by histological, immunohistochemical and morphometric studies.

MATERIALS AND METHODS

A) Drugs:

Aspirin: tablet form (Memphis Drug Company, Egypt), Each tablet contains 320 mg acetyl salicylic acid. It was given of a dose of 200mg\kg BW[3] dissolved in 0.5 ml 0.9% saline, to induce peptic ulcer[3].

Curcumin: powder form pure extract (Sigma Aldrich Company, St. Louis, Mo, USA). The required dose (40mg\kg BW)[13] was dissolved in 0.5 ml Dimethyl sulfoxide 20% (DMSO) (Abcam Company, UK).

Wild Honey: natural pure form (Royal Bees Stores, KSA) and the calculated dose (2g\kg BW)[7] was dissolved in 0.5 ml distilled water.

Omeprazole: capsules {Trade name (Pepzol MR)} (Hikma Pharma S.A.E company). Each capsule contains 20 mg omeprazole. The required dose (20mg\kg BW) [14] was dissolved in 0.5 ml 10% Tween solution (Sigma Aldrich Company, St. Louis, USA).

B) Animals:

This study included 30 adult male albino rats with average body weight 180-220 grams. They were housed in the Animal House of Kasr El Aini, Faculty of Medicine, Cairo University and treated in accordance to guidelines of the Animal Use Ethics Committee of Cairo University. Rats in all groups fasted 2 hours daily before giving any drug. All drugs were given orally by intragastric tube.

The rats in this study were divided into 2 main groups (n=15 each):

I. Group 1 (Control group): Received 0.5 ml saline 0.9% orally once daily for 6 days. Then the rats were equally subdivided into Subgroups 1a, 1b and 1c that received the solvent of the corresponding experimental group from day 6-10.

II. Group 2 (Experimental group): Received Aspirin once daily for 5 days. Then the rats were equally subdivided into:

a- Subgroup 2a (Untreated Group): continued Aspirin till day 10.

b- Subgroup 2b (Curcumin & Wild Honey Group): received a combination of curcumin and wild honey once daily from day 6-10.

c- Subgroup 2c (Omeprazole group): received Omeprazole once daily from day 6-10.

All rats were sacrificed on day 11. They were fasted overnight from food and fasted from food and water in the last 2 hours before scarification. They were anesthetized by intraperitoneal injection by phenobarbital (80mg\kg) [15] and stomach of each group was dissected, washed by saline and cut along the greater curvature and kept in 10% formol saline for 24 hours, processed and embedded in paraffin. Fundus and body sections were subjected to H & E stain, Periodic Acid Schiff's Reaction (PAS) and immunohistochemical staining using anti-Cox2 antibody.

For Immunohistochemical staining[16]: antigen retrieval was done using sodium citrate buffer (ph6) in a microwave oven for 2 minutes followed by cooling for 20 minutes. Then, anti Cox-2 monoclonal antibody (Lab vision, USA) was used at a dilution of 1:200) for 45 minutes at room temperature in humidity chamber. Then, ultravision universal detection system (HRP/DAB, Lab vision, USA) was used to detect the immunoreaction. This was formed of biotinylated anti-polyvalent secondary antibody, streptavidin peroxidase and DAB. The sections were then counterstained using Mayer's haematoxylin.

For quantitative and statistical analysis: The mean area percent of positive PAS reaction and of Cox-2 antibody immunopositivety were measured using the image analyzer computer system "Lecia Qwin 500 C" (Cambridge, UK). The data obtained were statistically analyzed by analysis of variance ANOVA test using "SPSS 17" software. P value < 0.05 were considered statistically significant.

RESULTS

Gross examination

In control group gastric mucosa was intact without any congestion, hyperemia or erosions Fig. (1a). Ulcer group showed hyperemia in most of the gastric mucosa denoting inflammation Fig. (1b). Both in curcumin and honey -treated group and in Omeprazole-treated group, marked decrease in mucosal hyperemia was observed Figs. (1c & 1d).

H&E- stained sections:

The histological results of the control subgroups were similar. The mucosa of the fundus showed thick mucus blanket over the surface epithelium, fundic glands opened into the lumen by short narrow gastric pits, they were very crowded with little amount of connective tissue in between and occupying most of the thickness of the mucosa. Surface mucous cells with basal oval nuclei were seen on the surface. In the middle part of the gland, large rounded oxyntic cells with deeply acidophilic cytoplasm and central rounded nuclei, as well as mucous neck cells with flat basal nuclei and pale foamy cytoplasm were seen (Figs. 2 a, b &c).

In the ulcer group, there was sloughing of the surface epithelium accompanied by loss of the mucous blanket and exfoliated cells in the lumen of stomach. Some glands showed distorted architecture and dilatation of lumen of the gland (Fig. 3a), some oxyntic cells were shrunken with pyknotic nuclei, others had pale vacuolated cytoplasm (Fig. 3b). Marked leucocytic infiltration was also observed (Fig. 3c).

The combined curcumin and honey-treated group showed a thick layer of mucus on the surface and surface cells were full of mucus with clear basal oval nuclei (Fig.4a). Most of oxyntic cells were normal with deep acidophilic cytoplasm (Fig.4b).

In omeprazole-treated group, fundic glands showed normal architecture and thick mucus layer on the surface. Mucous neck cells were full of faintly stained vacuolated mucus compressing the nucleus in the base. Also oxyntic cells had deep acidophilic cytoplasm and central rounded nuclei while there were still few oxyntic cells showing pyknotic nuclei and karyolysis Figs. (5a &b)

PAS stained sections (histogram 1):

The control group showed thick continuous PAS positive film of mucus on the surface and PAS positive reaction in the apical part of the lining surface cells. Moderately positive PAS reaction was seen in the mucous neck cells (Figs. 6 a &b).

In the ulcer group, an interrupted very thin PAS positive surface mucous film was observed. Moderate positive PAS mucous could be seen within the cells lining the gastric pit and in the hypertrophied markedly vacuolated mucous cells in the middle part of gastric glands (Figs. 7 a & b). Morphometric and statistical evaluation revealed a significant decrease as compared to the control group.

The curcumin and honey-treated group, showed thick PAS positive mucous film over the surface epithelium, strong PAS positive mucus in apical part of cells lining the lumen of gastric pits as well as in the mucous neck cell Fig.(8)

Histological examination of the omeprazole-treated group revealed a thick continuous PAS positive mucous film over the surface epithelium and strong PAS positive mucous in the apical part of cells lining the gastric pit, while the mucous neck cells contained moderately PAS positive mucous Fig.(9)

Both groups 2b and 2c showed a statistically non-significant difference as compared to control.

COX-2 immunostained sections (histogram 2):

The control group showed negative or minimal COX-2 immunoreaction on the surface, in the lamina propria between the glands Fig. (10).

The ulcer group showed strong positive immunohistochemical expression of COX-2 within some cells in the lamina propria at the ulcer bed Fig. (11a). Strong positive COX-2 immunoreaction appeared in endothelial cells lining the venules and capillaries as well as in many connective tissue cells in lamina propria. These cells were either rounded or elongated Fig. (11b). This reaction showed a statistically significant increase as compared to control.

In curcumin and honey-treated group Fig. (12) and in omeprazole-treated group Fig. (13), there was a negative or mild COX-2 immunoreactivity on the surface epithelium, fundic glands and in cells of lamina propria, that showed a non-significant difference when compared to control.
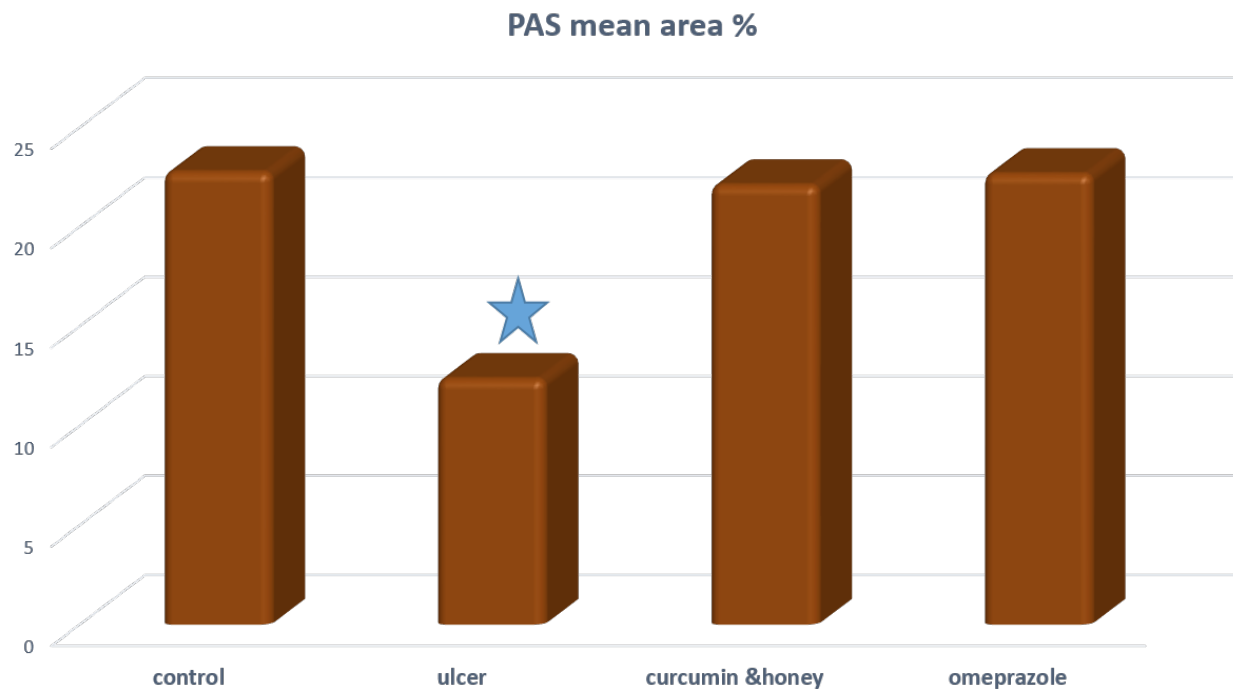
PAS mean area %

Histogram 1: Mean area % of positive PAS reaction in all tested groups.
* = significant as compared to control (P < 0.05)
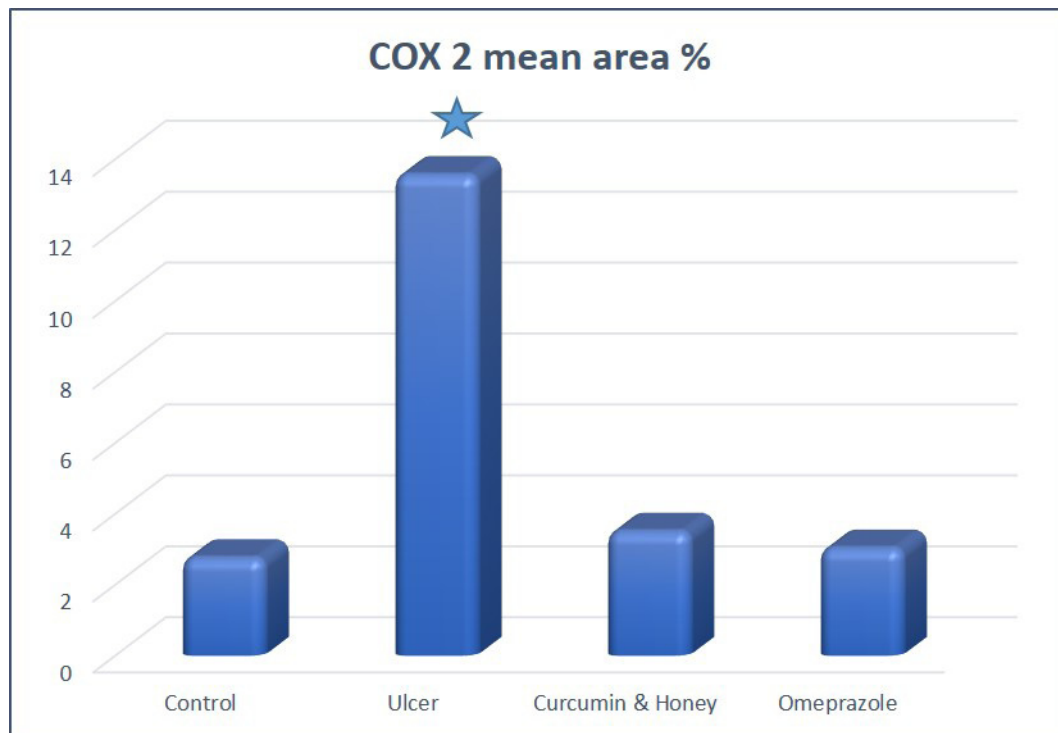
COX 2 mean area %

Histogram 2: Mean area % of Cox2 immunopositivity in all tested groups.
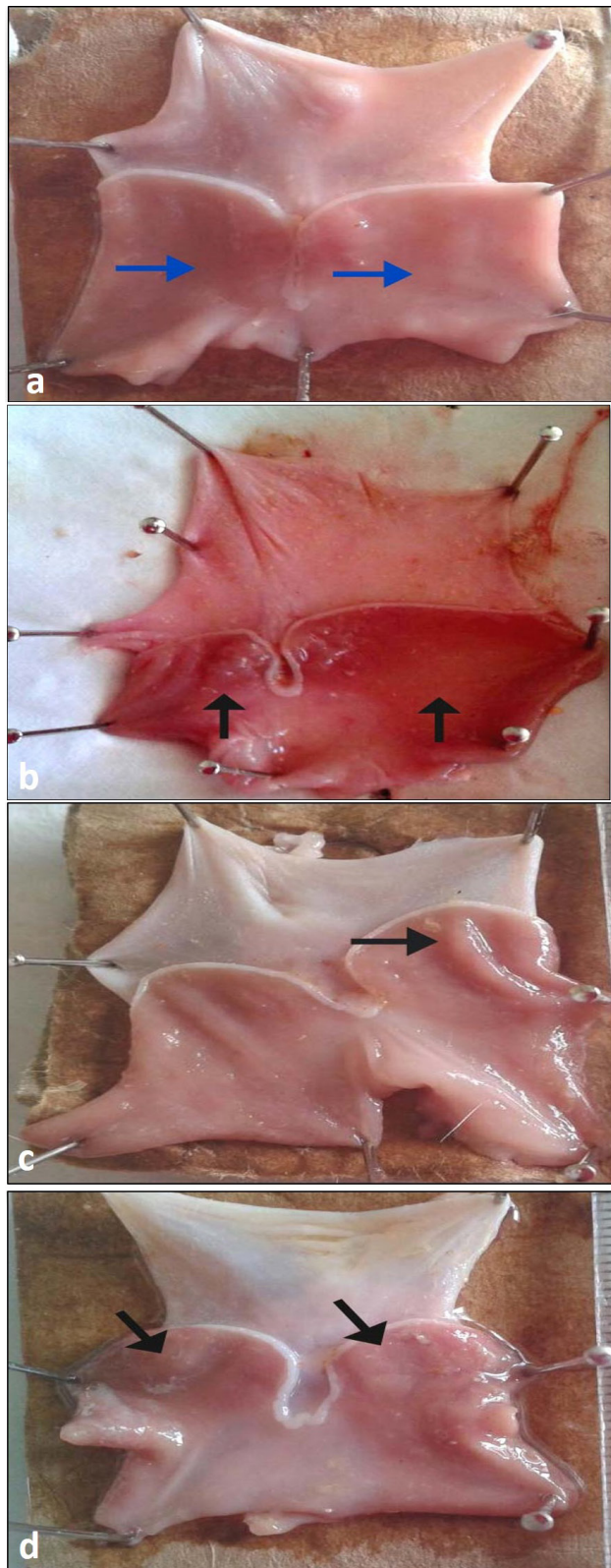***** = significant as compared to control (P < 0.05)

Fig. 1: Gross examination of the gastric mucosa showing: a) Control group: intact gastric mucosa (blue arrow). b) Ulcer group: hyperemia in most of the gastric mucosa (black arrow). c) Curcumin and honey-treated group: mild mucosal hyperemia (black arrow). d) Omeprazole-treated group: mild mucosal hyperemia (black arrow).
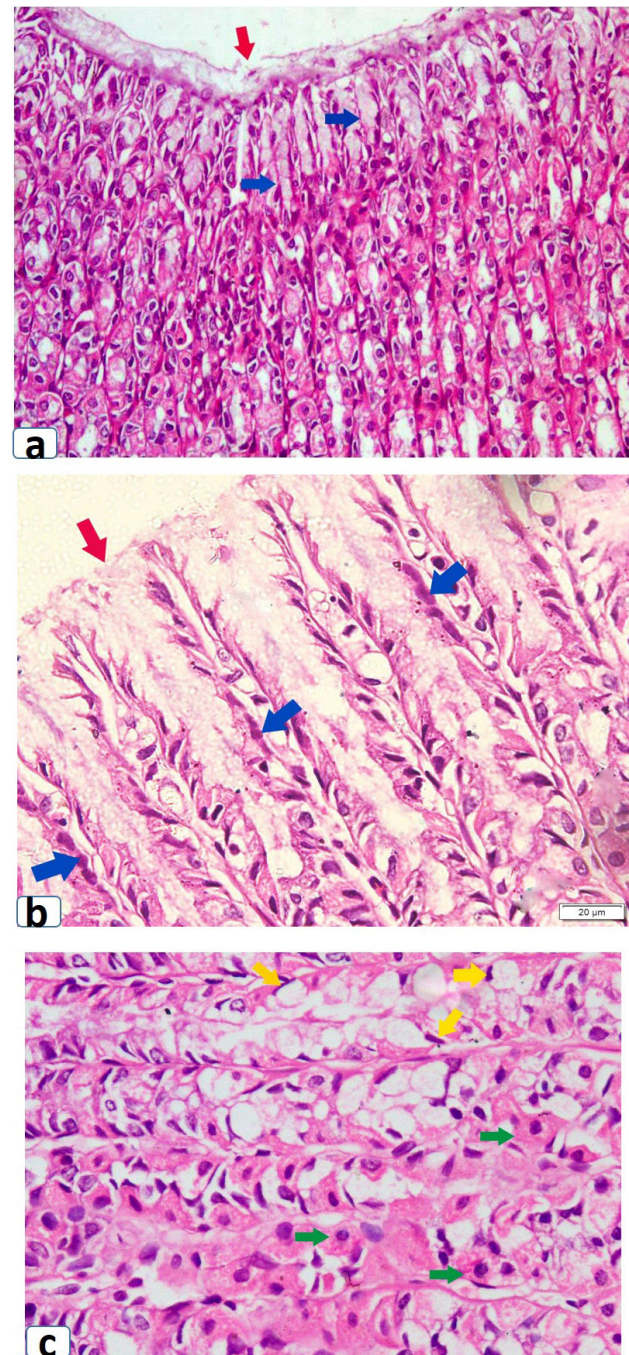

Fig. 2: Photomicrograph of fundic mucosa of a control group rat (group 1) showing: a) thick mucous film over the surface epithelium (red arrow), fundic glands open into the lumen by short narrow pits (blue arrow). b) High magnification of the upper part of the fundic glands with thick mucous blanket (red arrow) and normal surface mucous cells with basal oval nuclei (blue arrow). c) middle part of the gland with oxyntic cells having deeply acidophilic cytoplasm and central rounded nucleus (green arrow).Mucous neck cells with flat basal nuclei and pale foamy cytoplasm (yellow arrow) are seen. (H&E, a x 200, c & b x 400)
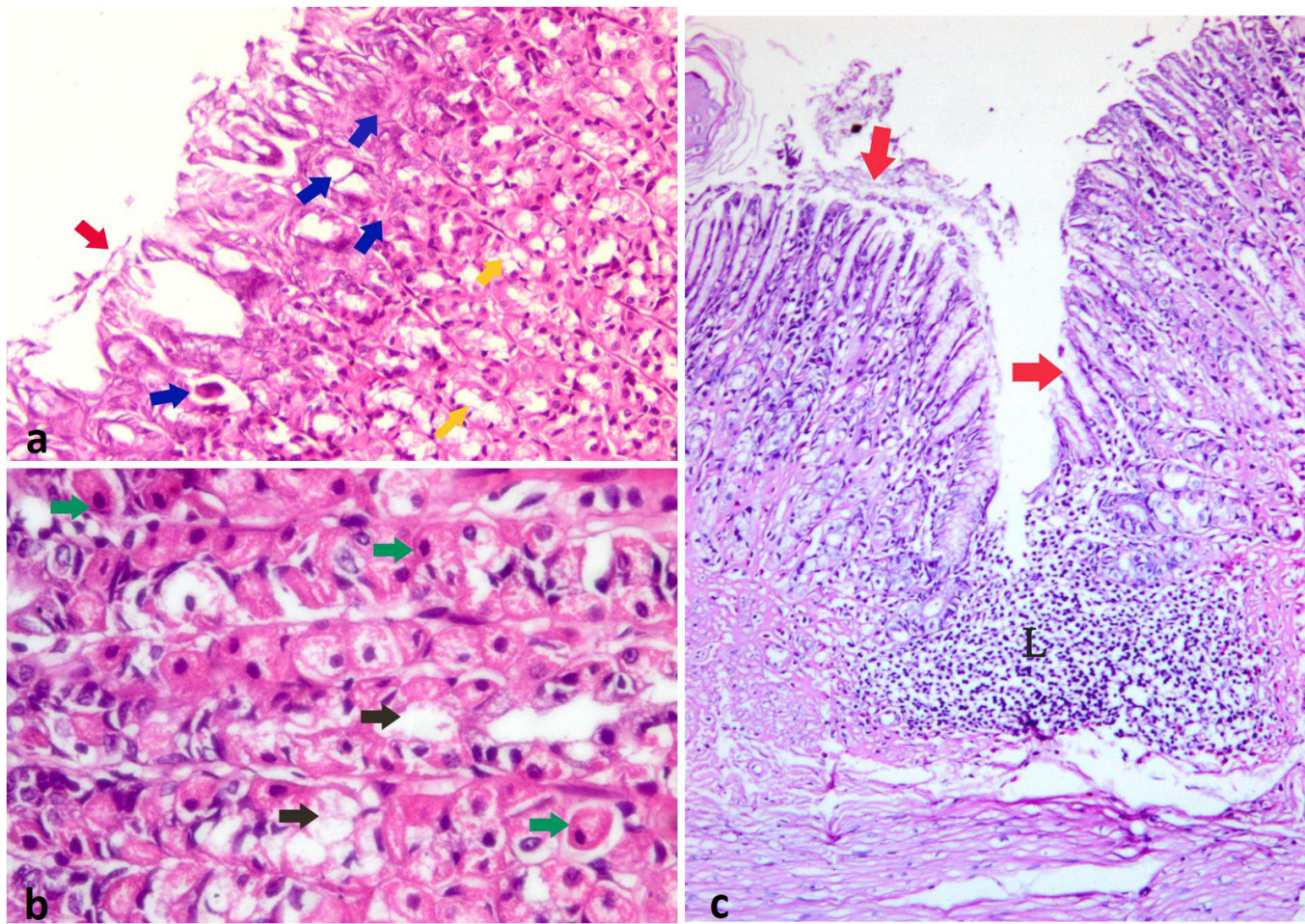
Fig. 3: Photomicrograph of fundic mucosa of an ulcer group rat (group 2a) showing: a) sloughing of the surface epithelium (red arrow), some glands show distorted architecture (blue arrow) with dilatation of their middle part (yellow arrow). b) Some oxyntic cells are shrunken with pyknotic nucleus while others are vacuolated (green arrow), there is also widening of gastric glands (black arrow). c) Sloughing of surface epithelium (red arrow) and marked leucocytic infiltration (L). (H&E, a x200, b x400, c x100)
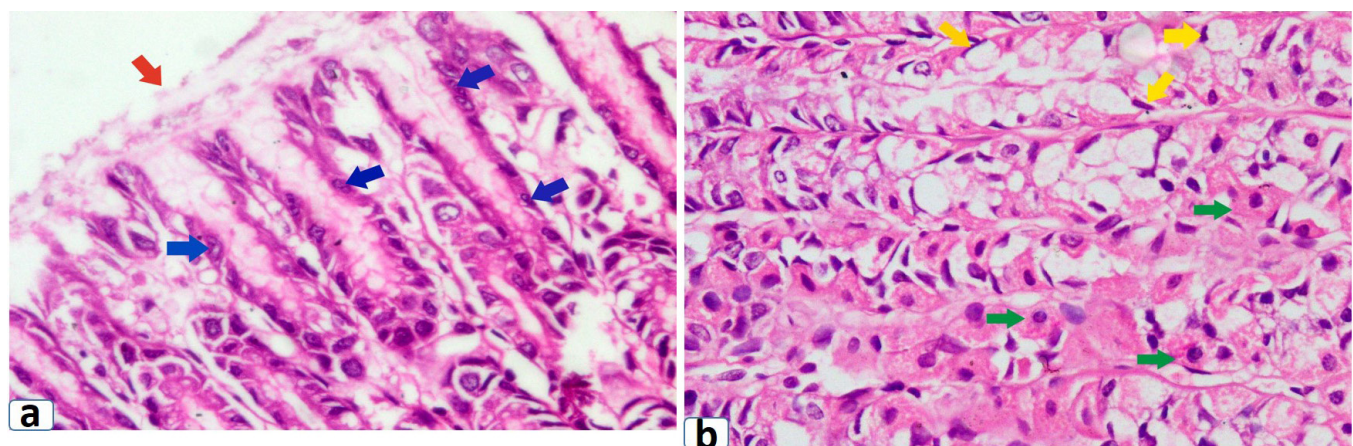

Fig. 4: Photomicrograph of the fundic mucosa of a group 2b rat showing: a) thick layer of mucus on the surface (red arrow) and surface mucous cells are full of mucus (blue arrow). b) Most oxyntic cells appear normal with deep acidophilic cytoplasm (green arrow) and mucous neck cells are full of mucus with basal oval nuclei (yellow arrow). (H&E, x 400)
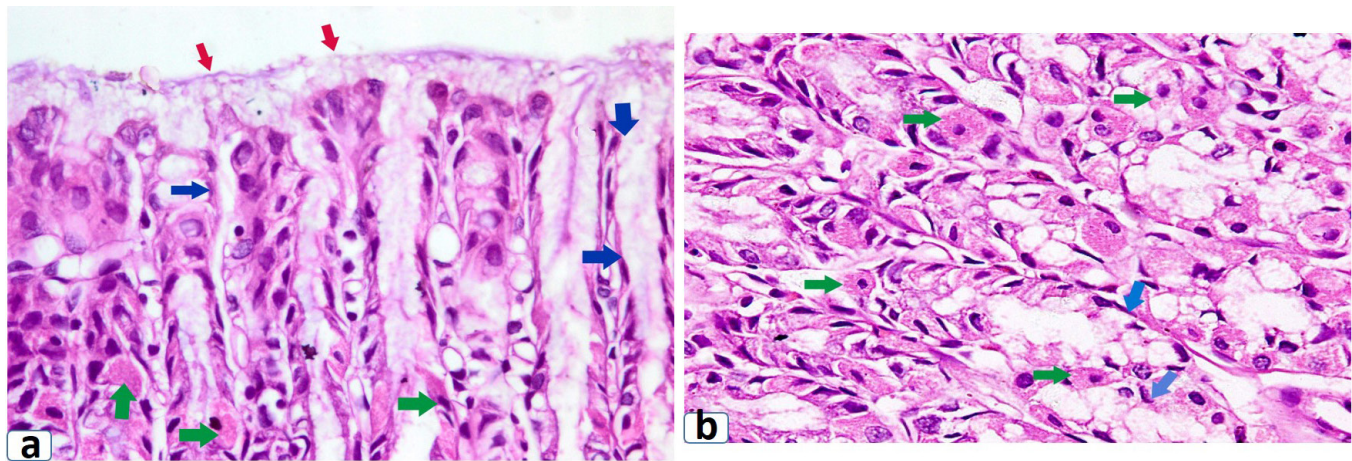
Fig. 5: Photomicrograph of fundic mucosa of an omeprazole group rat (group 2c) showing: a) Glands with normal architecture and thick mucous layer on the surface (red arrow), surface mucous cells appear normal and distended with mucus (blue arrow), while (green arrows) few oxyntic cells show karyolysis and others have pyknotic nuclei. b) Oxyntic cells with acidophilic cytoplasm and rounded nuclei (green arrow) and few cells show vacuolation and pyknotic nuclei. Mucous neck cells show normal appearance (blue arrow).   (H&E, x 400)
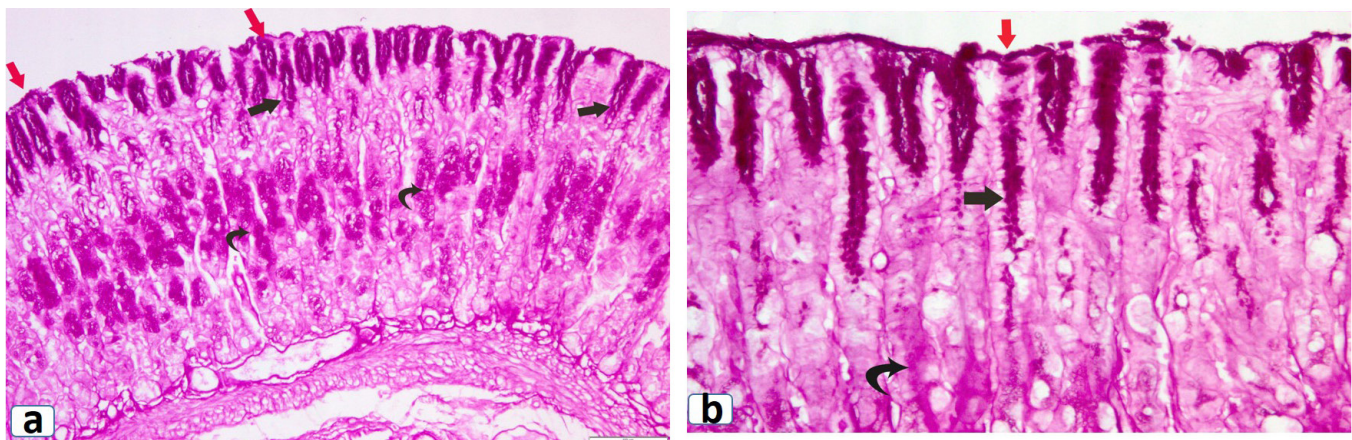

Fig. 6: Photomicrograph of fundic mucosa of a control group rat showing: a) PAS positive film of mucus on the surface (red arrow) and PAS positive mucus in surface mucous cells (black arrow). Neck region of the gland contains moderately positive mucus (curved arrow). b) thick continuous PAS positive film of mucus on the surface (red arrow) and PAS positive mucous in the apical part of the cells lining the gastric pit (black arrow). Also the neck region of the gland contains moderately PAS positive mucus (curved arrow) in the apex of the mucous neck cells.   (PAS a x 100, b x 200)
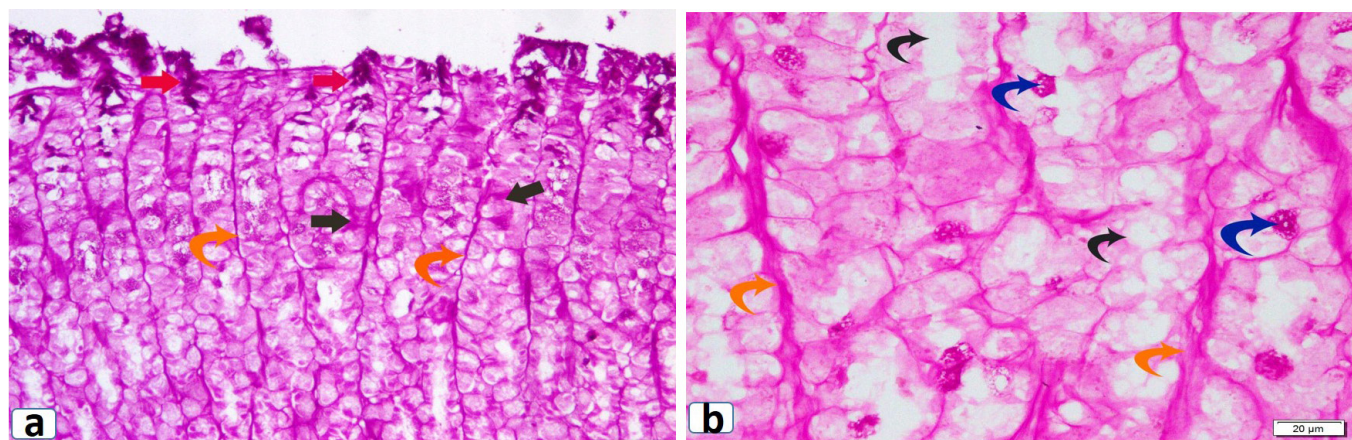

Fig. 7: Photomicrograph of fundic mucosa of an ulcer group rat (group 2a) showing: a) interrupted PAS positive reaction of surface mucous film (red arrow), moderate PAS positive mucus in cells lining the gastric pits (black arrow). Note the PAS positive basement membrane (orange curved arrow). 7b) markedly hypertrophied and vacuolated mucous cells lining the middle part of the glands (curved black arrow) and thickened basement membrane (orange curved arrow). Note the PAS positive reaction in the apex of some cells (blue curved arrow). (PAS a x 200, b x 400)
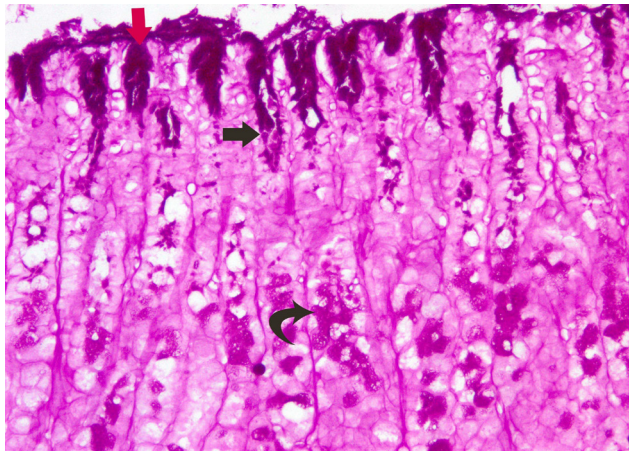
Fig. 8: Photomicrograph of fundic mucosa of a group 2b rat showing thick PAS positive mucous film over the surface epithelium (red arrow), strong PAS positive mucus in apical part of cells lining the lumen of gastric pits (black arrow). There is also a strong PAS positive reaction in the mucous neck cells (curved arrow).   (PAS x 200)
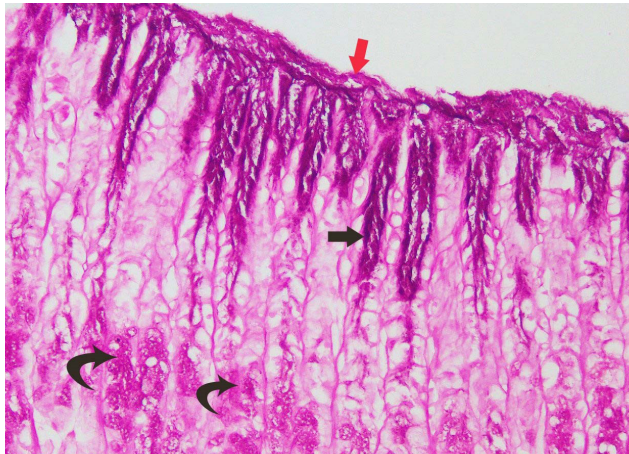

Fig. 9: Photomicrograph of fundic mucosa of a group 2c rat showing a thick continuous PAS positive mucous film over the surface epithelium (red arrow) and PAS positive reaction in the apical part of cells lining the gastric pit (black arrow). Mucous neck cells contain moderately PAS positive mucus (curved arrow).   (PAS x 200)
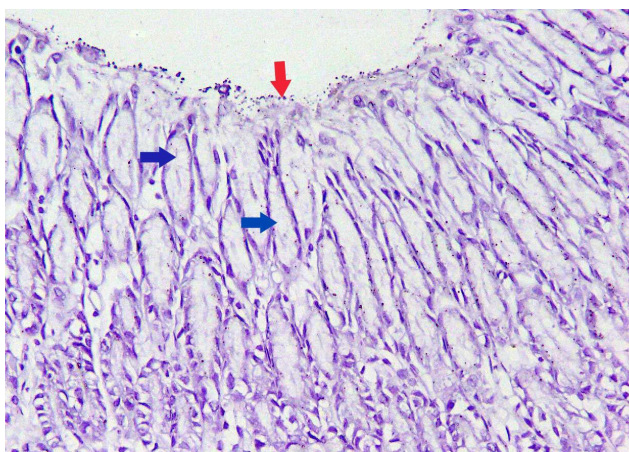

Fig. 10: Photomicrograph of fundic mucosa of a control group rat (group 1) showing negative COX-2 immunoreaction on the

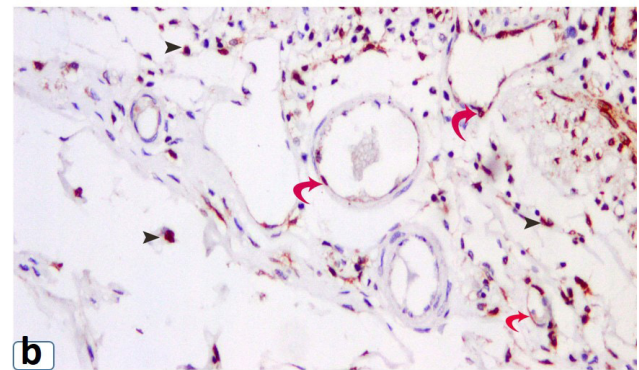surface (red arrow) as well as in the glands and lamina propria (blue arrow). (COX-2 x 200)
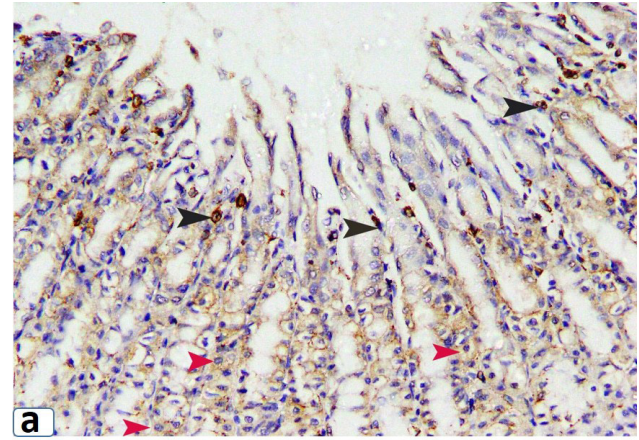
Fig. 11: Photomicrograph of fundic mucosa of a group 2a rat showing: a): Positive immunohistochemical expression of COX-2 within some cells in the lamina propria at the ulcer bed (black arrow head) and in the cytoplasm of some oxyntic cells in the middle part of the glands (red arrow head). b) Strong positive COX-2 immunoreaction in the endothelial cells (red curved arrow) lining the venules and capillaries in lamina propria and a strong reaction is seen in many connective tissue cells (arrowhead). Note that these cells are either rounded or elongated (COX-2 a x 200, b x 400)

Fig. 12: Photomicrograph of fundic mucosa of a group 2b rat showing negative COX-2 immunoreactivity on the surface epithelium (red arrow) and in between the glands (blue arrow). Note the thick continuous mucous layer on the surface (asterix). (COX-2 x 200)

Fig. 13: Photomicrograph of fundic mucosa of a group 2c rat, showing negative reaction for COX-2 in both surface epithelium (red arrow) and in lamina propria (blue arrow). (COX-2 x 200)

DISCUSSION
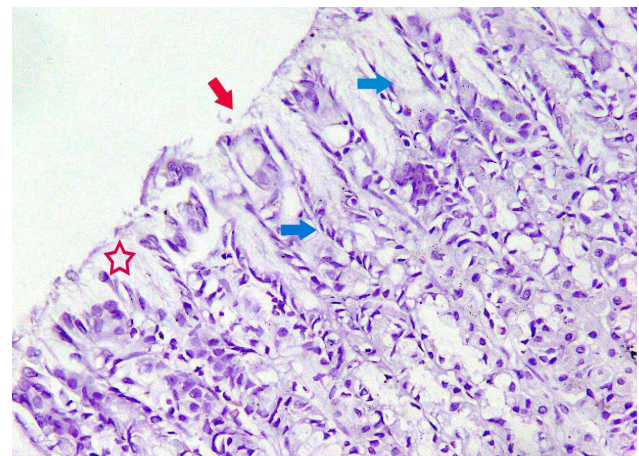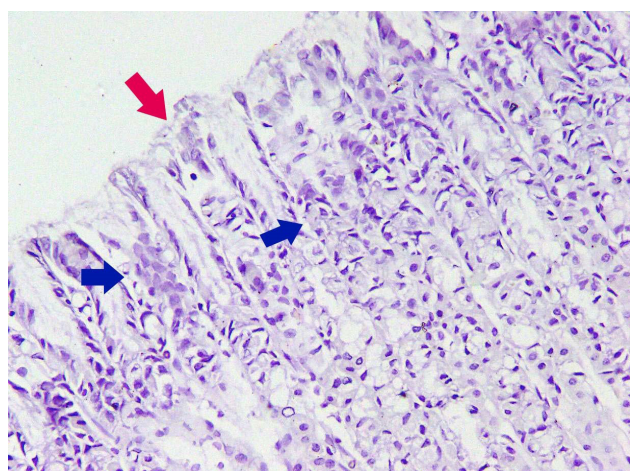
This work aimed to evaluate the possible effect of a combination of Curcumin and wild honey on aspirin-induced gastric in rats, as compared to omeprazole.

Gastric ulcer was produced by oral administration of aspirin for 10 days. This was evidenced by sloughing of surface epithelium, exfoliated cells and distorted fundic glandular architecture with dilatation of some gastric glands. These results were in agreement with another study[17] that reported superficial erosions in the gastric mucosa, remnants of the gastric glands appearing in the lumen, surface mucous cells with lightly stained cytoplasm and nuclear swelling, many mucous neck and chief cells with necrotic changes after oral administration of piroxicam (another NSAID) for 6 hours.

The mechanisms by which NSAIDS cause sloughing of the surface epithelium were proposed by Ittiyavirah and Paul (2016)[18], as they stated that their local action can directly kill epithelial cells which is attributed to their local inhibitory effect on gastric prostaglandin E2 (PGE2) and prostaglandin I2 (PGI2) that are the main inhibitors of gastric acid secretion. In addition, they induce osmotic lysis, subsequent to trapping of charged NSAIDs, leading to epithelial cells death due to uncoupling of oxidative phosphorylation. Also, NSAIDs have been reported to diminish the ability of epidermal growth factor (EGF) to promote epithelial repair, this appears to involve a reduction of EGF-binding to its receptor leading to inhibition of its signaling pathways with the subsequent inhibition of epithelial proliferation[19].

The dilated gastric glands that were detected in the current study could be due to the degeneration of cells lining these gastric glands giving the appearance of wide lumen[20].

In addition, the ulcer group also showed marked vacuolation and hypertrophy of the parietal cells while others appeared shrunken with pyknotic nuclei. These findings are in agreement with a study[21] that also demonstrated features of degeneration, in oxyntic cells of ulcer group. Their nuclei were shrunken with irregular nuclear membrane and excessive heterochromatin and apparent cytoplasmic vacuolations. These vacuolations could be related to the increased secretory canalicular membrane elaboration as a result of increased gastric acid secretion due to loss of the inhibitory effect of prostaglandins (PGs) on the oxyntic cells[22].

In the present work, marked ulcer-healing effects were detected in the combined curcumin and honey-treated group. Gastric glands regained their normal architecture with a thick layer of mucus on the surface, surface mucous cells and mucous neck cells were full of mucus and most of oxyntic cells appeared normal with deep acidophilic cytoplasm. These results were supported by other authors[7] who found that the erosion of mucosa and polymorph nuclear cellular infiltration, were markedly decreased in the combined curcumin and honey-treated groups.

Sections of the omeprazole-treated group showed normal glandular architecture, thick mucus layer on the surface, mucous neck cells full of mucous and normal appearance of oxyntic cells. These findings are in accordance with a recent study[23] that described omeprazole-treated ulcer group's sections with intact and normal-like gastric mucosa. In fact, omeprazole, a PPI, offered a reliable therapeutic drug, for its well established and widely proved ulcer-healing and gastro-protective effects[24]. Besides being a PPI, it is considered as an acid anti-secretory agent which has "direct" and "indirect" antioxidant activity and it also inhibits neutrophil activity[25].

In the current work, the PAS-stained sections of the aspirin-induced ulcer group showed a statistically significant decrease of PAS positive reaction, with a thin interrupted PAS positive surface mucous film and moderate PAS positive mucus in cells lining the gastric pits. Similar to those results, it was demonstrated[17] that ulcer group showed damaged gastric mucosa with marked decrease of the PAS positive mucus in the gastric pits and extending down into the lumen of the gland, while the neck region showed PAS positive mucus. They suggested that these changes were due to suppressed PGs production and damage of the surface epithelial cells and mucous neck cells which leads to decreased mucous production. The decrease in mucous secretion allows HCL and pepsin to diffuse into the mucosa from the lumen. Back-diffusion of acid and pepsin into the tissues stimulates further acid and pepsin secretion, decreases mucosal blood flow and decreases gastric motility. In the absence of normal PGs synthesis, the gastric environment becomes more vulnerable to exogenous or endogenous factors and, consequently, more prone to develop peptic ulcer[26].

In the same context, it was reported[27] that ulcerogenic agents cause dispersal of the gastro-protective mucous gel of the stomach and the associated phospholipids layers, leading to acid back diffusion and injury to the gastric mucosa. They added that oxidative stress associated with gastric ulcer results in lipid peroxidation that causes damage to the wall of the stomach.

In the same group, a remarkably strong PAS reaction was observed in the corrugated basement membrane. Several lines of evidence reported that the thickening of the basement membrane, associated with gastric ulcer, is related to the microangiopathy and microvascular proliferation and increased synthesis of basement membrane components[28]. Meanwhile, it was stated[29]that when the stomach is ulcerated, myofibroblasts reside beneath the epithelium then they migrate towards ulcer bed to form granulation tissue. In agreement with these results increased collagen fibers deposition between glands in the lamina propria was also observed[21]. The authors related this finding to the deleterious effects of aspirin which led to focal arteritis and fibrin deposition.

In the combined curcumin and honey-treated group, there was a thick PAS positive mucous film over the surface epithelium, in apical part of cells lining the lumen of gastric pits and in the mucous neck cell of neck region. Comparably, histological examination of the omeprazole-treated group showed thick continuous strong PAS positive mucus film over the surface epithelium and strong PAS positive mucus in the apical part of cells lining the gastric pits. Likewise, preserved gastric mucosal glycoprotein after omeprazole treatment was reported[23]. They explained that this could be attributed to its effect in increasing gastric NO level which leads to increased mucous secretion and reduced gastric acid secretion.

Morphological quantification of COX-2 immunopositivity, in ulcer group, showed the highest values. Positive COX-2 immunoexpression was detected within numerous cells in the lamina propria at and around the ulcer bed, as well as in the vascular endothelial cells, with a statistically significant increase when compared with the control group. In agreement with this, marked increase of COX-2 expression in the ulcer margin and in the ulcer itself was detected[30]. In accordance, it was observed[31] that COX-2 is barely detectable in normal gastric tissue in man, but is strongly expressed in the regenerating epithelium as well as in myofibroblasts, macrophages and endothelial cells within the granulation tissue, in gastric ulcer. They also observed that the cellular localization of COX-2 in human gastric ulcers was consistent with those reported in animal ulcer models. These findings are also concomitant with another study[16] that reported active inflammation in mucosa adjacent to gastric ulcer with intense COX-2 immunoreactivity in cells with the morphological appearance of macrophages, in myofibroblasts and in the vascular endothelial cells. These findings could be clarified

by another study[30] that related these results to the effect of COX-2 in generating PGs, especially at ulcer margin, which appears to play an important role in ulcer healing through triggering cell proliferation, promotion of angiogenesis and restoration of mucosal integrity.

It is worthy notice that, in the current study, COX-2 immunopositive cells, within the lamina propria, appeared either rounded or elongated. It could be hypothesized that the rounded cells could be inflammatory cells (most probably neutrophils and macrophages), while the elongated ones could be considered myofibroblasts.

This hypothesis could be lent further credence based on the work of Thong-Ngam et al. (2012)[32] who suggested that NSAIDs-induced gastric ulceration is a neutrophil-dependent process. They explained that NSAIDs administration to rats causes a rapid and significant increase in adhesion between neutrophils and vascular endothelial cells in both the gastric and mesenteric venules. These neutrophils release a variety of inflammatory mediators which are capable of producing tissue injury and might be involved in the pathogenesis of gastric mucosal injury. Furthermore, this could be supported by a recent report[33] stated that the inflammation induced in the gastric mucosa by aspirin is accompanied by increased TNF- production, which augments neutrophil derived superoxide generation and stimulates IL-1 production, leading to further neutrophil accumulation.

As for the implication of myofibroblasts, it was proposed[16] that myofibroblasts infiltration into the ulcer base is a prominent phenomenon seen in granulation tissue and are believed to be important in the process of revascularization of ulcer healing. High expression of COX in myofibroblasts may also promote cell proliferation. Concomitant with the findings of the present work, they also reported that human gastric endothelial cells expressed COX-2 in response to mitogenic stimuli and production of PGE2. They added that PGs synthesized by human gastric endothelial COX have potential to cause vasodilatation and angiogenesis.

Meanwhile, both in the combined curcumin and honey-treated group and in the omeprazole-treated group, there was negative or minimal COX-2 immunoreactivity on the surface epithelium and in between the fundic glands.

Accordingly, curcumin and honey combination possessed nearly similar effect in ulcer healing compared to the omeprazole-treated group, as shown by H&E, PAS and COX-2 immunohistochemical staining.

In accordance with these findings, it was revealed[7] that the combination of curcumin and honey effect is bigger than that of the curcumin alone in treatment of gastric ulcer, as it is believed that by consuming the combination of honey and herbs, the therapeutic outcome could be more effective than by using it alone.

Since both curcumin and honey share an important mechanism as anti-inflammatory substances through down-regulating the activity of COX-2, while, curcumin alone inhibits lipoxygenase, iNOS enzymes and inflammatory cytokines[34], and honey alone reduces PGs and thromboxane B2[35], it could be proposed that the use of both agents together could have a synergistic effect, that yielded comparable therapeutic efficacy to that of omeprazole.

Accordingly, it could be concluded that, in this rat model of aspirin-induced ulcer, combined curcumin and honey therapy proved to have a therapeutic potential in improving fundic stomach alterations. This provides experimental evidence ensuring the satisfactory effect of this combination compared to omeprazole treatment, offering a reliable alternative therapeutic measure for gastric ulcer without any adverse effects of drugs. Inhibition of COX-2 pathway is effective in restoration of stomach structure after combined curcumin and wild honey treatment of gastric ulcer. However, further studies are required to uncover the exact therapeutic mechanism of this combination, and to elicit its possible prophylactic effect of on gastric ulcer.

POTENTIAL CONFLICT OF INTEREST

The authors have no conflicting financial interest

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