Original Article

# Comparative Study on the Ameliorative Effect of Indian Costus Extract Versus Omeprazole on Fundic Mucosa in Ulcer Model

*Sara Adel Hosny Mohamed, Mira Farouk Youssef Yacoub, Alaa Essam-Eldin Fasseh and Amal Elham Ahmed Fares*

*Department of Histology, Faculty of Medicine, Cairo University, Cairo, Egypt*

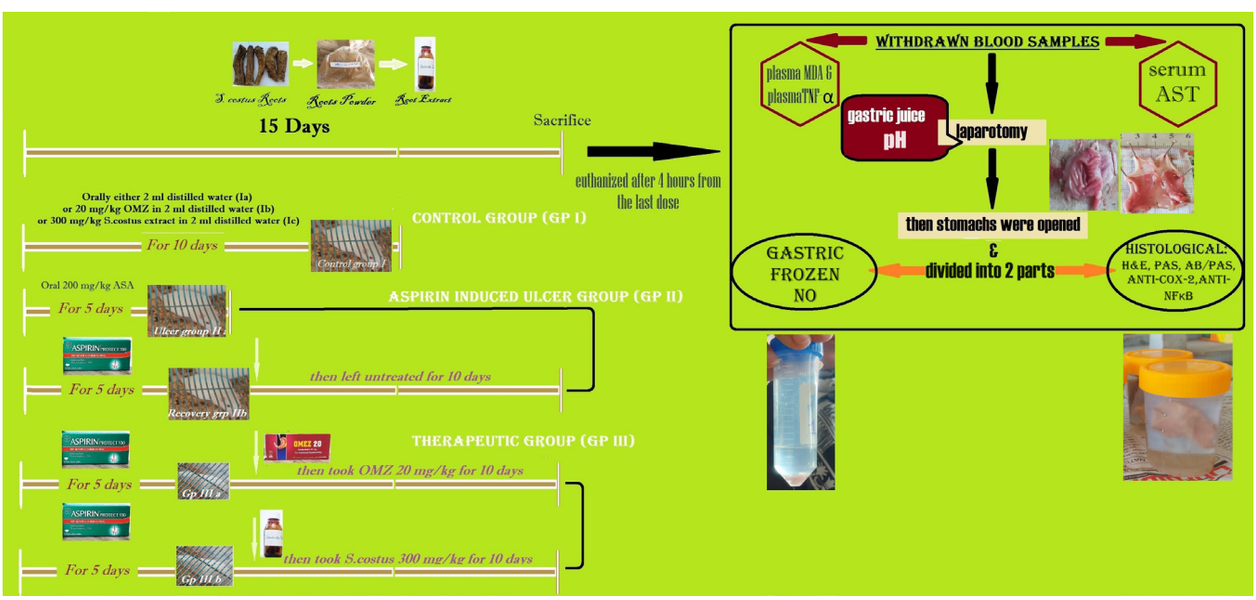
## ABSTRACT

**Introduction:** Gastric peptic ulcer disease is of major global health burden which lead to serious complications if left without treatment. This study aimed at evaluating the ameliorative effect of Indian costus ethanolic extract versus omeprazole as the most recently used ulcer therapy in aspirin induced peptic ulcer.

**Materials and Methods:** 29 rats were divided into: Group I (Control group) which is sub divided into three subgroups (distilled water, omeprazole control that recieved omeprazole for 10 days, saussurea control that recieved saussurea for 10 days), Group II: subgroup IIa (Aspirin; ASA diseased), subgroup IIb (Recovery), Group III: subgroup IIIa (omeprazole; OMZ), subgroup IIIb (saussurea costus; S costus). Aspirin was given daily orally for 5 days to all groups. After 5 days, group IIIa received Omeprazole, group IIIb received S costus for 10 days. Serum malondialdehyde (MDA), tumor necrosis factor alpha(TNF-α), Aspartate aminotransferase (AST) and frozen gastric nitric oxide (NO), prostaglandin E2 (PGE2) were measured. Fundic sections were subjected to histological. immunohistochemical and morphometric analysis.

**Results:** Subgroups (IIa & IIb) revealed marked loss of gastric lining with cells having dark pyknotic nuclei. A significant increase in mean values of MDA, TNF-α, AST, mean area % of Cycloxygenase-2 (COX2) & Nuclear factor Kappa-B (NFκB) immunopositivity with decrease in mean area % of (periodic Acid Schiff) PAS in group IIa & IIb compared to other groups. Omeprazole and S costus treatment ameliorated the biochemical, histological and immunohistochemical changes induced by aspirin on fundic mucosa.

**Conclusion:** Aspirin can lead to metaplastic changes on the cellular level in the histological structure of fundic mucosa. The S costus produced comparable results to omeprazole with significant improvement in fundic mucosa in gastric ulcer rat model and improvement in the liver function parameters.




**Graphical Abstract**

**Received:** 31 March 2024, **Accepted:** 11 June 2024

**Key Words:** Aspirin intestinal metaplasia, COX-2, NFκB, omeprazole, saussurea costus extract.

**Corresponding Author:** Alaa Essam Eldin Ali Fasseh, PhD, Department of Histology, Faculty of Medicine, Cairo University, Cairo, Egypt, **Tel.**: +20 11 4277 2517, **E-mail:** alaaessam123@gmail.com / alaa.fasseh@cu.edu.eg

**ISSN:** 1110-0559, Vol. 48, No. 2

*DOI: 10.21608/ejh.2024.275752.2046*

## INTRODUCTION

Peptic ulceration (PU) is defined as an insult to the digestive mucosa resulting in ulceration extending from mucosa to the submucosa. Sites of peptic ulcers (PUs) include the stomach, duodenum and occasionally esophagus[1]. Gastric ulcer usually occurs with cigarette usage, nonsteroidal anti-inflammatory drugs (NSAIDs), or alcoholism[2]. It affects four million people worldwide yearly[3], causing significant morbidity and mortality worldwide[4].

In gastric mucosa, there are many cytoprotective factors including prostaglandins (PGs), mucosal bicarbonate, nitric oxide (NO) and oxidative antagonist system. Proper blood supply is thus a protective mechanism for the gastric mucosa[5]. There are aggressive agents that interfere with the protective factors including acid, pepsin, bile regurge, abnormal motility, decreased blood flow and bacterial invasion with Helicobacter pylori. The imbalance between mucosal defense mechanisms and the opponent agents results in gastric ulcer development[6].

Different diseases include aspirin which is a powerful nonsteroidal anti-inflammatory drug (NSAID) as part of the therapy such as rheumatoid arthritis and its related diseases. It is also used in cardiovascular prophylaxis against thrombosis. Aspirin usage leads to a major problem which is gastric ulcer[7]. Patients with previous history of peptic ulcer disease are subjected to bleeding with NSAID use[4].

Perforation, bleeding, and occlusion are consequences of peptic ulcer disease. Although perforation is second to bleeding in frequency, it is a surgical emergency with morbidity of 50% and a fatality rate of 30% of patients[8]. Complications of gastric ulcer may progress to gastric cancer with high morbidity and mortality rates[2].

The peptic ulcers treatment has been revolutionized lately by the proton pump inhibitors (PPIs) as Omeprazole that reduces gastric acidity[9]. All PPIs have been considered relatively safe drugs[10]. They are part of the gastro-esophageal reflux and peptic ulcer therapy[11]. They act on the H+/K+ ATPase proton pump in the parietal cells and prevent H+ ions secretion. This results in inhibiting acid secretion and consequently elevating gastric pH[12]. However, PPIs have many adverse effects including headache, diarrhea, abdominal pain, nausea, hypomagnesemia, and hyponatremia. They may also cause proliferative changes, acute interstitial nephritis, increased risk of pneumonia and calcium deficiency fracture[13].

Herbal medicine has a critical role in alternative and traditional medicine, which is used to treat many different diseases. It can be easily obtained, inexpensive and overall, it fits individuals' social and cultural needs[14]. Indian costus (Saussurea costus) is an important medicinal plant containing many active ingredients like flavonoids, steroids, costunolide, lactone dehydrocostus, phenols, and glycosides[15]. Saussurea costus is believed to have several biological activities like antiulcer, anti-inflammatory, anti-bacterial and antifungal actions[16]. Saussurea costus has been used for treatment of various illnesses such as fever, bronchitis, rheumatoid arthritis, typhoid fever, and chronic skin diseases[17].

This work was done to observe the possible ameliorative effect of Indian costus versus omeprazole on aspirin induced gastric ulcer in male adult albino rat model and suggest the underlying mechanisms through histological, immunohistochemical, morphometric and biochemical methods.

## MATERIALS AND METHODS

### *Drugs*

**Aspirin:** (Aspirin®protect100) tablets, (each containing 100 mg acetyl salicylic acid) was bought from Memphis Co. Pharmaceuticals and Chemicals Industries (Egypt).

**Omeprazole:** (Omez®) capsules, (each containing 20 mg), was obtained from Pharaonia Pharmaceuticals (PHAROPHARMA) (Alexandria, Egypt).

### *Herbal*

**Saussurea costus:** The alcoholic extract was extracted from the root of Saussurea costus (S costus): The dry roots of Saussurea costus were purchased from local herbal market (Egypt) in the form of powder.

**Preparation of ethanolic extract from Saussurea costus:** 200 grams of powder were extracted with ethyl alcohol at room temperature soaked for 24 h. Watman No. 1 filter paper was used to purify the extract then concentrated under vacuum using a rotary evaporator[18]. This process was performed in the Biochemistry Department. Dose preparation: extract powder was divided into equal amounts of 300 mg suspended in 26 ml distilled water. Each rat was given 2 ml daily for 10 days.

### *Experimental Design*

This study included twenty-nine male albino adult rats aged 12 weeks, with an average body weight of 170-200 grams. Rats were housed in Kasr Al Ainy House of Animals and dealt with in accordance to guidelines of the Ethical Committee of Animal usage.

The rats were divided randomly into three groups: each group was kept in separate wire cages at RT and kept under constant day/night cycle in a climate-controlled condition with an access to food and water ad libitum. Rats were deprived of food 2 hours before receiving any drug daily.

**Control group (Gp I):** 9 Rats were divided into 3 sub-groups:

Distilled water control subgroup (Ia): (3 rats) Each received 2 ml distilled water orally / day for 10 days.

Omeprazole control subgroup (Ib): (3 rats) Each received 20 mg/kg omeprazole (≈4mg in 2ml distilled

water/rat) orally dissolved in 2 ml distilled water daily for 10 days.

Saussurea costus control subgroup (Ic): (3 rats) Each received 300 mg/kg Saussurea costus extract orally dissolved in 2 ml distilled water daily for 10 days.

**Aspirin induced ulcer group (Gp II):** 10 rats were divided into 2 subgroups:

Diseased subgroup IIa: (5 rats) Each received aspirin at an oral daily dose of 200 mg/kg for 5 days to induce peptic ulcer then euthanized after 4 hours from last dose[19]. It was daily prepared by crushing aspirin tablet suspending it in distilled water and each rat received 0.5 ml of the solution[7].

Recovery subgroup IIb: (5 rats) Each received aspirin at an oral dose of 200 mg/kg/day for 5 days to induce peptic ulcer and left untreated for 10 days then euthanized at the end of experiment.

**Therapeutic group (Gp III):** (10 rats) Each received aspirin as Gp IIa to induce peptic ulcer then they were divided into 2 subgroups:

Subgroup IIIa: (5 rats) Each received 20 mg/kg omeprazole orally dissolved in 2 ml distilled water daily for 10 days[20].

Subgroup IIIb: (5 rats) Each received 300 mg/kg saussurea costus extract orally dissolved in 2 ml distilled water daily for 10 days[15].

Rats of all groups were mercifully sacrificed using an intraperitoneal (IP) injection of phenobarbital (200 mg/kg)[21]. Blood was then extracted into capillary tubes from the retroorbital sinuses and split into two portions. The first was separated from the serum by centrifuging it at 4000 rpm for 15 minutes. The serum was then kept at -80° until it was needed to measure serum aspartate aminotransferase (AST). The remaining blood samples were placed in vials containing EDTA and centrifuged for 15 minutes at 4°C at 1800 rpm to extract serum for the measurement of tumor necrosis factor-α (TNF-α) and malondialdehyde (MDA).

After a laparotomy, the stomachs were removed, and the contents were taken to measure the pH of the gastric juice. To get rid of any debris, gastric tissue specimens were gently rinsed with phosphate buffer saline (PBS). Every stomach was split into two sections. The first section was homogenized in PBS after being delivered as a sample in ice-cold PBS (0.1 M, pH 7.4) to the biochemistry department. The supernatant was then extracted from the homogenates by centrifuging them for five minutes at 5000 rpm and 4 °C. In order to perform a biochemical assay for gastric nitric oxide (NO), the supernatant was frozen and kept at -80 °C[22].

For a full day, the remaining portion of the stomach was fixed in 10% formalin[23]. After that, it underwent processing and paraffin embedding to create tissue blocks for histological analysis. Fundus and body sections were cut at 5 µm thickness and stained histologically (hematoxylin and eosin for routine examination of the cells and nuclei), histochemically (PAS to stain mucopolysaccharides, and Alcian PAS to distinguish neutral mucin from acidic one) and immunohistochemically (COX-2 for inflammation ,NFkB as an oxidative DNA damage marker).

### *Immunohistochemical staining*

1. Anti-cyclooxygenase (COX-2) antibody[24]: It is a rabbit monoclonal IgG antibody Clone SP21 (In*vitro*gen, Thermo Fisher Scientific Laboratories, USA, catalogue number: MA5-14568).
2. Anti- Nuclear factor Kappa-B (NFκB)[25]: (Epredia™, Lab Vision, Thermo Fisher Scientific Laboratories, USA; catalogue number: RB-9034-P) is a rabbit polyclonal IgG antibody.

Sections were deparaffinized in xylene and then rehydrated using progressively lower alcohol grades. For 15 minutes, the slices were submerged in $H_2O_2$ to prevent endogenous peroxidase activity. The sections were incubated with two drops of primary antibody for 60 min after 5 minutes of blocking non-specified background with Ultra V Block. After that, incubate the slides for ten minutes in two drops of biotinylated goat anti polyvalent secondary antibody and then for ten minutes in two drops of streptavidin-peroxidase. The reaction was visualized using the Ultravision One detection system, HRP polymer, and diaminobenzidine (DAB) Plus Chromogen. The slides were washed with xylene, dried with increasing alcohol concentrations, counterstained with Mayer's hematoxylin, and mounted. The +ve reaction appeared as deep brown cytoplasmic reaction for inflammatory marker COX-2 Ab and as brown-reddish fine granules located in the cytoplasm and progress to nuclear reaction for NFκB Ab as an oxidative DNA damage marker.

### *Biochemical Assays*

**The venous blood samples were used for determination of**

- The concentration of serum AST, using commercial kits (NS Biotec Company, catalogue number: GOT-MC-02100) in U/L[26] to exclude liver affection.
- Serum MDA (as an oxidative stress marker) in nanomole (nmol)/ml)[26] using commercial kits (Biodiagnostic Company, catalogue number: MD 25 28).
- Serum TNF- α (as an inflammatory marker) in picogram (pg)/ml[19] using commercial rat kits (CUSABIO Company, catalogue number: CSB-E07967r).

**Gastric frozen section for**

Measurement of gastric mucosal nitric oxide (NO) (as gastric mucosal protective factor) in µmol /gram protein using commercial kits (Biodiagnostic Company, catalogue

number: NO 25 32). Gastric NO content was indicated by the nitrite level. Measurement of nitrite take place through reducing nitrate to nitrite and was followed by addition of Griess reagent[27].

Both were measured according to the manufacturers' instructions by ELISA assay in in Biochemistry Department, Faculty of Medicine.

**Gastric juice sample**

The gastric contents collected were centrifuged in a centrifuge tube for 10 minutes at 1000 rpm to remove any solid remnants. The pH of this solution was recorded from the taken supernatant using a pH meter in the in Biochemistry Department, Faculty of Medicine.

### *Morphometric study*

Obtained data was analyzed by "Leica Qwin 500C" computer image analyzer system. (Leica Imaging System Ltd, Cambridge, UK). 10 non-overlapping fields were captured from each slide. The following parameters were measured:

- Sections stained with PAS: the mean area percent (%) of +ve reaction was assessed at a magnification of (x200) in 10 non-overlapping fields.
- In immunohistochemical stained sections:
    - the mean area % of COX-2 Ab IE were assessed at a magnification of (x200) in 10 fields.
    - the mean area % of NFκB Ab IE were calculated at a magnification of (x200) in 10 fields.

All were assessed using binary mode.

### *Analytical statistics*

The statistical package for social science (SPSS) software, version 16 (SPSS, Chicago, USA), was used for the analysis. The one-way analysis-of-variance (ANOVA) and post hoc Tukey test were used to compare the groups. The findings were presented in the form of mean ± standard deviation (SD). Statistical significance was determined by looking at differences with a probability ($p$) value less than 0.05[28].

## RESULTS

### *Histological findings*

**Hematoxylin and Eosin stain**

Normal mucosa with no major histological differences were observed in fundic sections stained with H&E when comparing control subgroups (Ia & Ic) apart from congested blood vessels seen in omeprazole control subgroup (Ib) (Figure 1).

The ulcer subgroups (IIa&IIb) showed distortion with reduced epithelial thickness, and wide pits. Many mucous cells with dark nuclei, and vacuolated cytoplasm were seen. Oxyntic cells were either shrunken deeply acidophilic with dark nuclei or hypertrophied with vacuolated cytoplasm and few were acidophilic with central nuclei. Most peptic cells were dark shrunken with dark nuclei or pale vacuolated and few peptic cells were pyramidal basophilic with rounded nuclei. Mild inflammatory cell infiltrations were also found with many vacuolations and separations in lamina propria and submucosa (Figure 2: A to D are diseased subgroup (IIa) while E to I are recovery subgroup (IIb)).

The therapeutic subgroups (IIIa&IIIb) showed epithelium regaining thickness, closely packed fundic glands but with some dilatation seen. Some atypical surface mucous and mucous neck cells appeared with dark nuclei while others showed normal shape. Also, there were many oxyntic cells with deeply acidophilic cytoplasm and central rounded pale nuclei. Some oxyntic cells were hypertrophied with vacuolated cytoplasm; others were shrunk with dark nuclei. At the base some peptic cells were apparently normal with deeply basophilic cytoplasm and basal rounded nuclei, others appeared distorted shrunken deeply stained with dark nuclei or with vacuolated cytoplasm. Leucocytic infiltration was evident in the lamina propria and some vacuolations were seen (Figure 3: A&B are omeprazole treated while C D& E S costus treated).

**Staining with periodic acid-Schiff (PAS)**

Examination of the control groups showed thick PAS positive (+ve) film of mucous on the surface, PAS +ve reaction in apical part of the surface epithelial cells that reached down to the pits of the glands. Also, the neck region contained a moderate PAS +ve reaction in the mucous neck cells. Omeprazole control subgroup (Ib) had a weaker reaction on the surface down the pits with decreased reaction in the neck region. Examination of the ulcer-induced and recovery group revealed weak PAS positive reaction down the pits with focal loss of PAS reaction at the eroded surface epithelium and focal loss of the reaction in neck region.

The omeprazole therapeutic group revealed a continuous PAS +ve mucous film in many regions, moderate PAS +ve reaction at the healed regions of the gastric glands at the apical part of surface epithelial cells, cells lining the pits and weak reaction in the neck cells. On the other hand, S costus therapeutic group showed thick PAS +ve mucous film over most of the epithelial surface, PAS +ve reaction in apical part of cells at the surface and lining the lumen of the pits as well as in the neck cells (Figure 4).

**Alcian blue/PAS (AB/PAS) stain**

Examination of the control groups showed thick positive (+ve) mucous film and +ve reaction of the surface epithelial cells reached down the pits of the glands where the acidic mucin stained purple to blue, Also, the neck region contained AB negative -ve, moderate PAS +ve magenta reaction in the mucous neck cells as neutral

mucin is stained magenta. Omeprazole control subgroup (Ib) had a weaker reaction with AB -ve, PAS +ve magenta film. Examination of the ulcer-induced and recovery group revealed weak +ve reaction down the pits with focal loss of reaction at the eroded surface epithelium and focal loss of the reaction in neck region. There were patterns of reversed reaction in recovery group IIb with focal AB +ve base reaction. The intestinal metaplasia was confirmed by the AB +ve staining with cup appearance of the goblet cells on higher magnification.

The omeprazole therapeutic group revealed a continuous PAS +ve mucous film in many regions at the apical part of surface epithelial cells, cells lining the pits and weak reaction in the neck cells. there was +ve AB reaction noticed at the base of the glands. On the other hand, S costus therapeutic group showed thick mucus film over most of the epithelial surface, +ve reaction in apical part of cells at the surface and lining the lumen of gastric pits as well as in the neck cells and -ve reaction at the base (Figure 5).

## *Immunohistochemical Results*

### COX-2 immune stained sections

Examination of control group showed +ve cytoplasmic immunoreaction of COX-2 mainly in the middle part of the glands and weak +ve reaction in the connective tissue CT could be seen. OMZ control subgroup (Ib) had more diffuse cytoplasmic immunoreaction of COX-2 throughout the mucosa with +ve expression in the blood vessels lining in the CT. Ulcer group exhibited obvious strong +ve cytoplasmic immunoreaction of COX-2 on the surface epithelial lining of the glands with +ve reaction in the CT cells. Therapeutic groups revealed moderate to faint +ve cytoplasmic immunoreaction of COX-2 among the epithelium mainly at the base of the glands. Some +ve immunoreaction was detected in the CT cells and the lining of some blood vessels (Figure 6).

### NFκB immune stained sections

The control subgroups (Ia,Ic) revealed negative nuclear and cytoplasmic NFκB expression in most of the cells. In addition, occasional cells had +ve nuclear immunoreaction. While Omeprazole control subgroup (Ib) had more cells with +ve nuclear immunoreaction. Aspirin administration caused strong cytoplasmic and nuclear NFκB immunoreaction throughout the gastric mucosal cells. Omeprazole therapeutic subgroup exhibited many cells with moderate cytoplasmic immunoreaction and many cells with +ve nuclear immunoreaction throughout the fundic mucosa. The S costus therapeutic subgroup sections revealed fundic mucosa with moderate to faint cytoplasmic immunoreaction in many cells. Occasional cells with +ve nuclear immunoreaction were noticed (Figure 7).

## *Biochemical Results*

### Gastric juice pH and NO results

By observing the pH values in all groups, it was found that the OMZ control subgroup (Ib) displayed a statistically significant increase ($P > 0.05$) compared to control subgroups (Ia,Ic), whereas the ulcer diseased (IIa) and recovery (IIb) subgroups exhibited a significant decrease in pH associated with significant dramatic increase of mean values of gastric NO compared to all other groups. A significant increase of gastric PH with decrease of gastric NO were found in therapeutic (IIIa, IIIb) groups when compared to ulcer (IIa, IIb) group. Therapeutic groups revealed nonsignificant differences when compared to control group (Histogram 1,2).

The MDA and TNF-α values indicated a statistically significant increase ($P<0.05$) in ulcer groups compared to all other groups and in the diseased subgroup (IIa) in TNF-α values when compared to the recovery subgroup (IIb). On the other hand, there was a statistically significant decrease ($P<0.05$) in both parameters in therapeutic group in comparison with ulcer (diseased and recovery) groups. Surprisingly, there was a significant increase in OMZ therapeutic subgroup (IIIa) when compared to control group. Meanwhile the S costus therapeutic subgroup showed nonsignificant difference in MDA values when compared to control group and significant increase in TNF-α values when compared to control subgroups (Ia,Ic). S costus therapeutic subgroup revealed nonsignificant decrease in MDA values but TNF-α showed a significant decrease when compared to OMZ therapeutic group (Histogram 3,4).

The serum AST levels showed a statistically significant increase in ulcer group (diseased IIa and recovery subgroup IIb) compared to all other groups. A significant increase in AST levels was found in OMZ therapeutic subgroup versus control subgroups (Ia, Ic). Whereas S costus therapeutic subgroup exhibited nonsignificant difference to control group in AST values. Finally S costus when compared to OMZ in therapeutic group showed a statistically significant decrease in AST levels (Histogram 5).

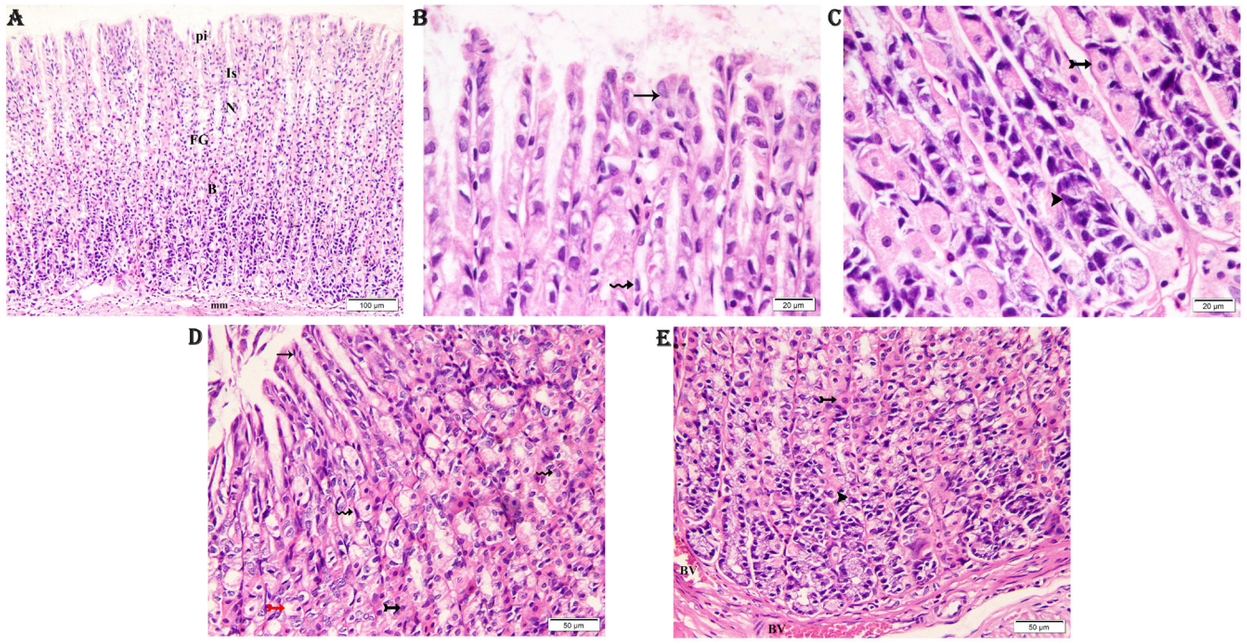



**Fig. 1:** A: control subgroup (Ia) showing closely packed tubular fundic glands (FG), perpendicular to the surface occupying the whole thickness of the lamina propria. Fundic glands are composed of isthmus (Is), neck (N) and base (B). Notice the pits (pi) and muscularis mucosa (mm). (B,C): higher magnification showing the surface mucous cells (arrow) appear full of mucous with basal oval nuclei, mucous neck cells (wavy arrow) in the pit having pale foamy cytoplasm, with basal flattened nuclei. Oxyntic cells (bifid arrow) appear as large, rounded cells in the middle and base of the gland with deep acidophilic cytoplasm and central pale vesicular nuclei. At the base of the gland, the peptic cells (arrowhead) are pyramidal with deep basophilic cytoplasm and rounded nuclei. (D,E): omeprazole control group showed few vacuolated oxyntic cells with dark nuclei (red bifid arrow). Notice congested blood vessels (BV).
(H&E, (A) x100, (B,C) x400, (D,E) x200)

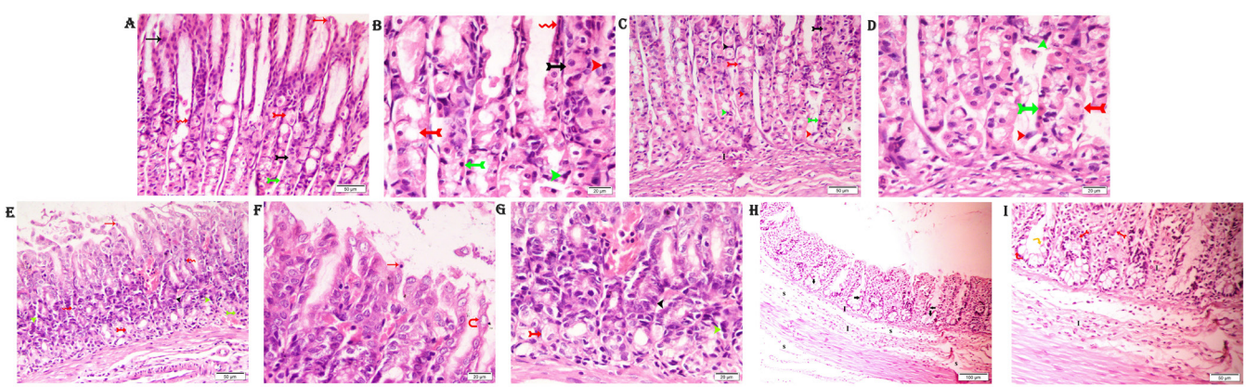



**Fig. 2:** The ulcer group showing surface mucous (red arrow) and mucous neck (red wavy arrow) cells are either shrunken deeply acidophilic with dark nuclei. Most oxyntic cells are either shrunken with dark nuclei (green bifid arrow) or expanded with vacuolated cytoplasm (red bifid arrow) and few show deep acidophilic cytoplasm and central pale vesicular nuclei (O). Peptic cells are distorted in shape and either deeply stained with dark nuclei (green arrowhead) or vacuolated (red arrowhead). The recovery subgroup (IIb) revealing more affection with nuclear margination (red curved arrow) seen at higher magnification. (H): A recovery subgroup (IIb) section showing markedly diminished thickness of the gastric epithelium with complete architecture destruction. Epithelial lining is lost and replaced by epithelium close to intestinal epithelium appearance in crypts (arrow). The lamina propria, and submucosa show separations (s) and infiltrated with many mononuclear leucocytes (I). Fig 2 (I) is a higher magnification showing many goblet-like cells (red kinked arrow) with basal flat nuclei and mucous apically are seen and some cells are columnar with apical brush border (orange kinked arrow). Occasional oxyntic cells (red bifid arrow) with acidophilic cytoplasm can be identified. Lamina propria and submucosa showing separations (s) and many leucocytic infiltrations (I).
(H&E, [(A to D): diseased subgroup IIa, (E to I): recovery subgroup IIb], (A,C,E,I) x200, (B,D,F,G) x400, (H) x100)

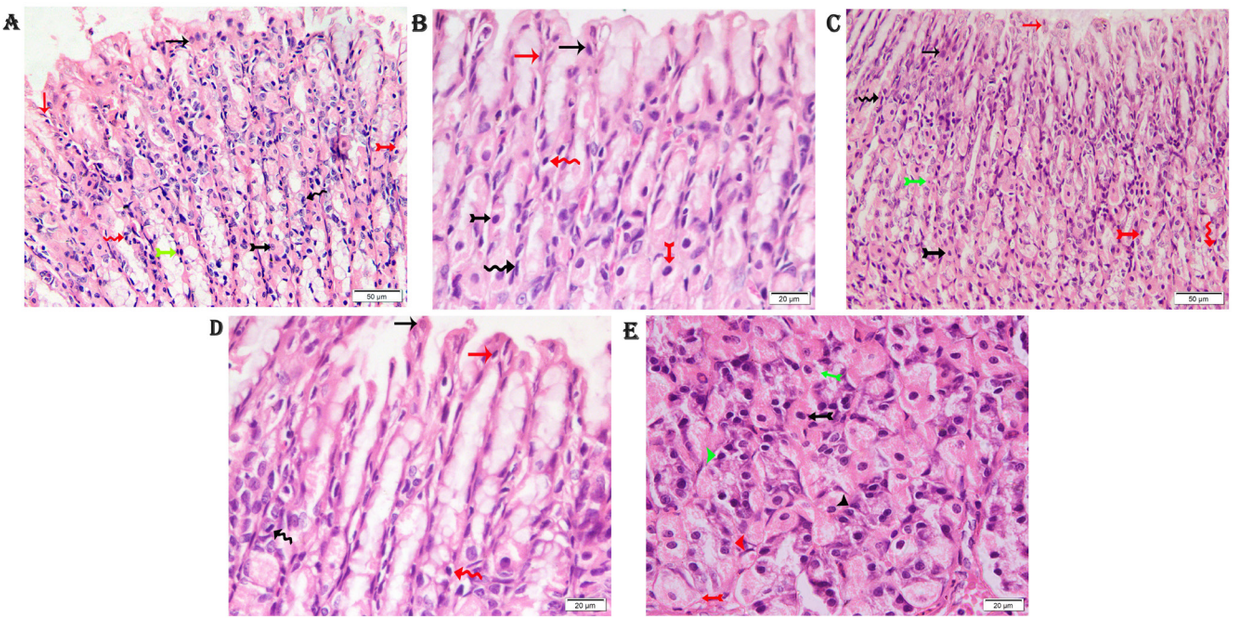



**Fig. 3:** revealing therapeutic group with the middle part of the glands seen dilated with some surface mucous cells (red arrow) and mucous neck cells (red wavy arrow) seen with dark nuclei. There are many surface mucous (arrow) cells with basal oval nuclei and mucous neck (wavy arrow) cells with basal flattened nuclei. Also, many oxyntic cells (bifid arrow) exhibit deeply acidophilic cytoplasm and central rounded pale nuclei and some are seen shrunken with dark nuclei (green bifid arrow), others with pale vacuolated cytoplasm (red bifid arrow) in omeprazole subgroup (IIIa) Figs 3(A, B) while, less affected cells can be noticed in S costus subgroup (IIIb) in (C, D, E). (H&E, (A,C) x200, (B,D,E) x400)

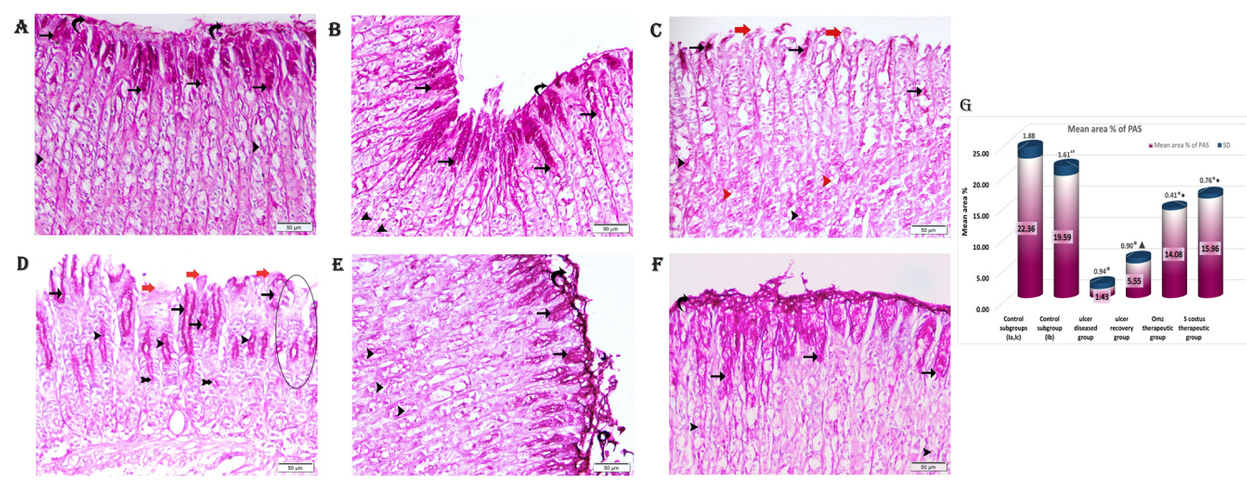



**Fig. 4:** A photomicrograph showing PAS positive (+ve) thick film on the mucosal surface (curved arrow), +ve reaction in the apical part of surface mucous cells reaching down to pits (arrows) and +ve reaction in the mucous neck cells in neck region (arrowheads). The mucus film is slightly thinner in omeprazole control group. In ulcer group, there is loss of PAS reaction at the surface epithelium down the pits (red arrows), scanty +ve PAS reaction (arrows) in other areas and focal loss of the reaction in neck regions (red arrowheads) of the fundic glands more obvious in recovery with +ve PAS reaction near the basal region (the bifid arrows) of the fundic glands. Notice pattern of loss of reaction at the upper part with +ve reaction near the lower region of the gland (circle). The Histogram: mean area % of PAS showing the improvement in therapeutic group compared ulcer group. Notice the omeprazole control subgroup compared to other control subgroups and recovery subgroup versus diseased group. (G): Histogram for mean area % of PAS: *significant compared to control group, ‘’significant compared to control subgroups (Ia,Ic), ● significant compared to ulcer group and ▲significant compared to ulcer diseased subgroup.
((A): control subgroup (Ia), (B): omeprazole control subgroup (Ib), (C): diseased subgroup IIa, (D): recovery subgroup IIb, (E): Omeprazole therapeutic subgroup, (F): S costus therapeutic subgroup. PAS, (A to F) x200)

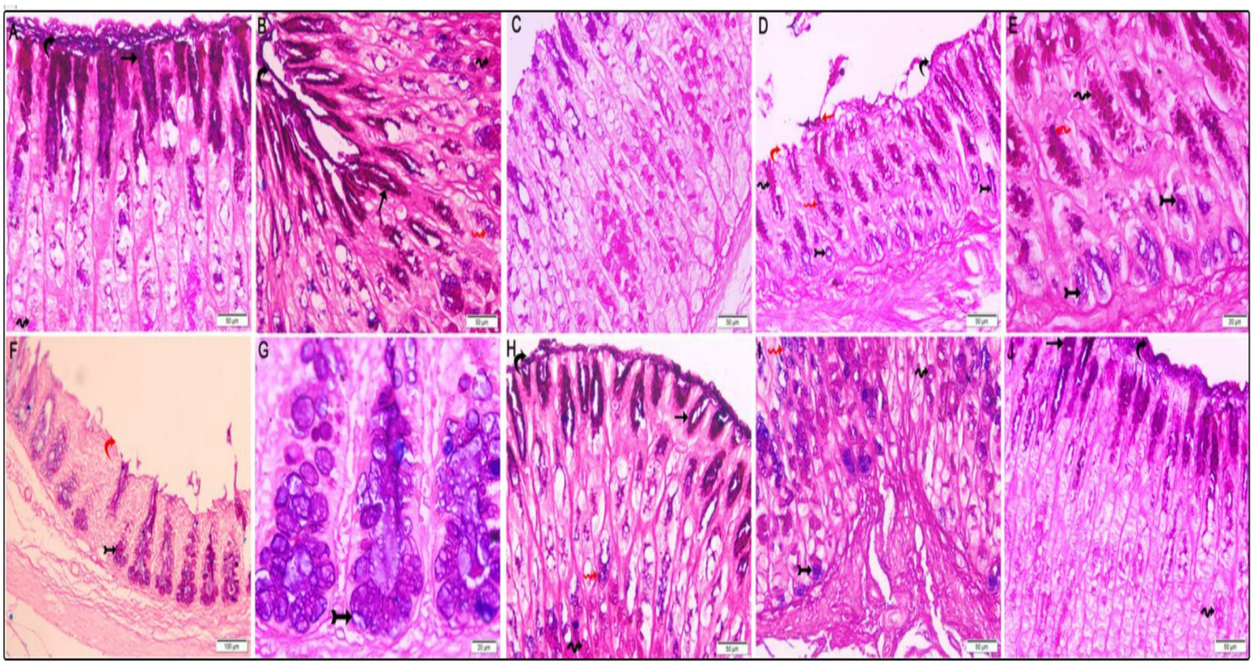


**Fig. 5:** The intestinal metaplasia as seen in (F, G) stained purple by Alcian blue (AB +ve). Note the weak reaction in diseased group IIa and patterns of reversed reaction in recovery group IIb with AB +ve reaction at the base marked by bifid arrow, there is +ve base reaction noticed in OMZ ttt group. {the mucous blanket (curved arrow), surface mucous cells (straight arrow), mucous neck cells (wavy arrow), +ve reaction at the base (bifid arrow)}
((A): control subgroup (Ia), (B): omeprazole control subgroup (Ib), (C): diseased subgroup IIa, (D,E): recovery subgroup IIb, (F,G): Intestinal metaplasia in recovery subgroup, (H,I): Omeprazole therapeutic subgroup, (J): S costus therapeutic subgroup. AB/PAS, ([A to D] & [H to J]) x200, (F) x100, (E,G) x400)

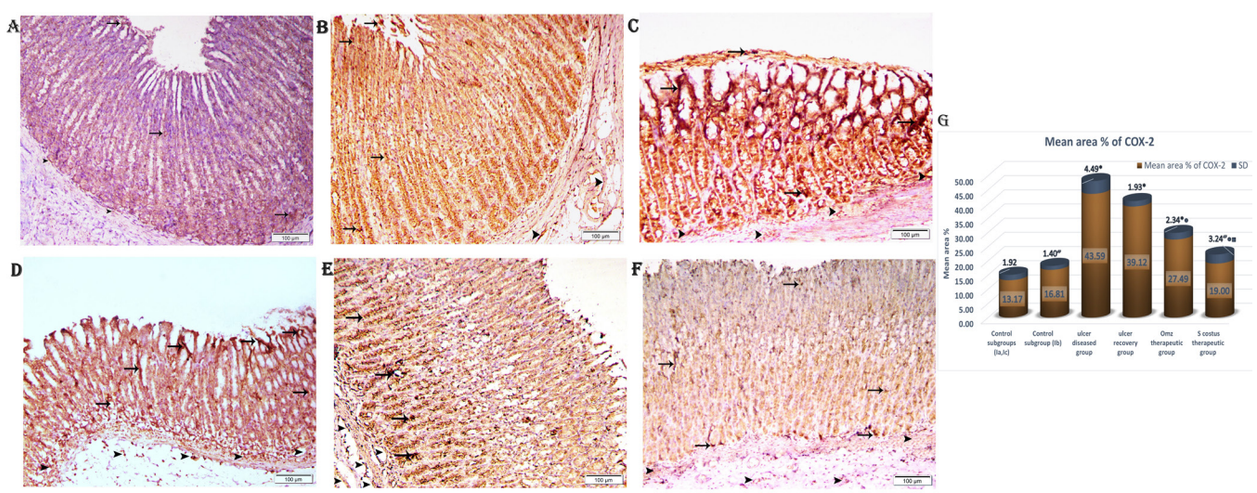



**Fig. 6:** A photomicrograph of fundic sections showing +ve cytoplasmic immunoreaction of COX-2 mainly at the middle part of the glands (arrows) and +ve reaction can be seen in the CT (arrow heads). The omeprazole control subgroup (Ib) showed reaction at the upper part of the glands and in bloods vessels in the CT. The reaction in the ulcer group is more diffuse involving almost all the thickness of the glands. The therapeutic group showing moderate +ve immunoreactivity which was fainter in the S costus therapeutic subgroup (IIIb). (G): Histogram for mean area % of COX-2: *significant compared to control group, ‘’significant compared to control subgroups (Ia,Ic), ● significant compared to ulcer group and ■ significant compared to OMZ therapeutic subgroup.
((A): control subgroup (Ia), (B): omeprazole control subgroup (Ib), (C): diseased subgroup IIa, (D): recovery subgroup IIb, (E): Omeprazole therapeutic subgroup, (F): S costus therapeutic subgroup. COX-2 immunostaining, (A to F) x100)

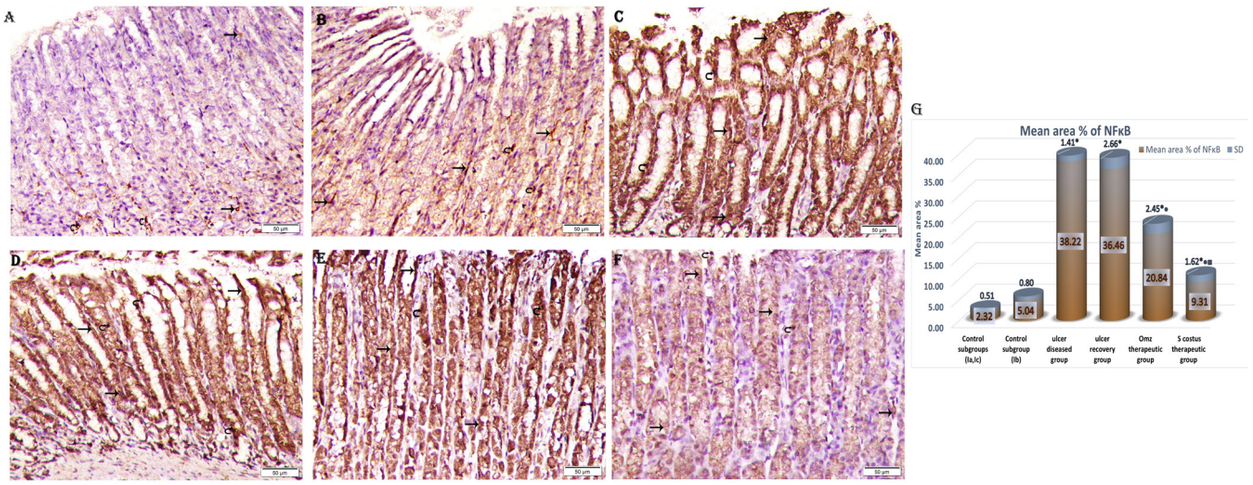
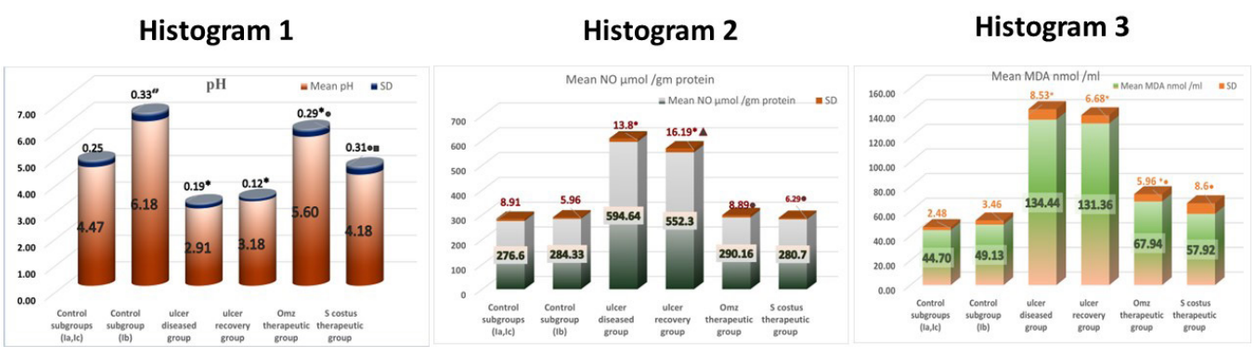
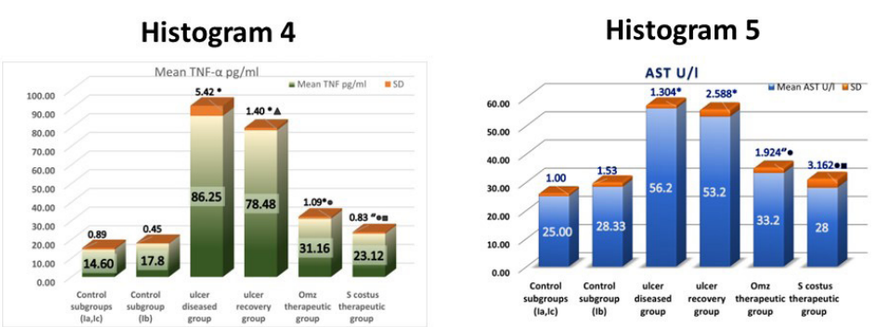



**Fig. 7:** A photomicrograph of a fundic section in control subgroup (Ia) showing cytoplasmic immunoreaction (arrow) and +ve nuclear reaction (curved arrow) the reaction is faint in control group, more obvious and diffuse in the ulcer group, and moderate positivity in the therapeutic group with better improvement in the S costus therapeutic subgroup (IIIb). (G): Histogram for mean area % of NFκB: *significant compared to control group, ‘’significant compared to control subgroups (Ia,Ic), ● significant compared to ulcer group and ■ significant compared to OMZ therapeutic subgroup.
((A): control subgroup (Ia), (B): omeprazole control subgroup (Ib), (C): diseased subgroup IIa, (D): recovery subgroup IIb, (E): Omeprazole therapeutic subgroup, (F): S costus therapeutic subgroup. NFκB immunostaining, (A to F) x200)




**Histograms:** Biochemical results expressed as Mean ± standard deviation (SD), Histogram 1: pH, 2: Mean nitric oxide (NO) in gastric frozen section, 3: Mean serum malondialdehyde (MDA) , 4: Mean serum tumor necrosis factor-alpha (TNF-α), 5: Mean serum Aspartate aminotransferase (AST). *significant compared to control group, ‘’significant compared to control subgroups (Ia,Ic), ● significant compared to ulcer group, ▲significant compared to ulcer diseased subgroup, and ■ significant compared to OMZ therapeutic subgroup

## DISCUSSION

Peptic ulcer is a worldwide chronic disease which impairs the quality of life. It is associated with mucosal break greater than 3-5 mm, may be accompanied by visible submucosal injury. The imbalance between the aggressive (gastric acid secretion) and defensive (gastric mucosal continuity) factors lead to gastric ulceration[29]. The current gastric ulcer treatment with anti-secretory drugs, such as proton pump inhibitor (PPI), H2 antagonist and antacids, on the long run had serious adverse problems such as gastric or intestinal effects, rebound hypergastrinemia, allergic reactions, hepatorenal toxicity, and arrhythmias[30].

Botanical drugs are raising the attention for their effectiveness, security, affordability, and suitability for use with human systems. Numerous investigations have confirmed that certain plant extracts have gastroprotective properties[31].

The current study compared the ameliorative effect of S costus versus omeprazole on aspirin induced fundic ulcer in adult male Wistar rat model. This was evidenced by histological, immunohistochemical, morphometric and biochemical methods.

The fundus and the stomach's body were chosen in accordance with earlier studies that claimed parietal cells predominated there, making these areas the most frequently affected by gastric ulcers[19].

Several biochemical markers showed significant changes in this study. The pathological process was found to be multifactorial. The current investigation revealed a statistically significant rise in the average serum TNF-α, MDA and gastric NO expression among ulcer group (Gp IIa and Gp IIb) when compared to all other groups. TNF-α as a proinflammatory cytokine induces inflammation accompanied by increased cytotoxic NO leading to reactive oxygen species (ROS) rise and oxidative stress appeared in MDA results. This agrees with previous study which stated that TNF-α (a predictive marker of apoptosis and inflammation) modulated apoptotic gastric cell death through caspase-3 pathway where gastric cell growth and angiogenesis are suppressed by the caspase-3 pathway, at the ulcer margin delays healing[32]. A study reported that the NFκB activation in their ulcer group promoted the TNF-α expression[33].

Concomitantly, a study mentioned that ROS rise led to gastric erosion and ulcer genesis. MDA is an oxidative stress marker generated by lipid peroxidation of polyunsaturated fatty acids (PUFA) in cell membranes and it accompanies increased ROS[34]. Another study confirmed that MDA can create intra- and intermolecular cross-links with proteins that cause apoptosis. It is harmful to cells[35]. Controversially, other studies clarified that the main source of ROS in the induced gastric injury is activated neutrophils infiltration producing ROS that react with cell lipids, forming lipid peroxides, which eventually appear as MDA rise[36].

In addition, NSAIDs may inhibit oxidative phosphorylation in the mitochondria, which causes the release of cytochrome C from the mitochondria and the ROS release further increasing lipid peroxidation. Lipid peroxidation alters the composition of the membrane and exacerbates stomach ulcers[33]. ASA can also increase the ROS, superoxide and can alter cellular cAMP concentrations. NSAIDs increase the adhesion molecules expression with WBCs attachment to gastric vascular epithelium obstructing blood flow leading to microvascular ischemia with free radicals' formation[37].

Since NO breaks down quickly into nitrite and nitrate, the quantity of nitrite/nitrate in the stomach mucosa is recognized as a marker of NO generated as a result of NOS activity[38]. NSAIDs induced the upregulation of iNOS genes in the stomach mucosal cells, which resulted in the large-scale release of NO. Large levels of peroxynitrite, a highly reactive nitrogen species that damages stomach mucosal cells, are produced when the NO reacts with superoxide anions (nitrosative stress)[39].

Going with our study, previous reports that recorded nitrite/nitrate dramatical increased in ulcer induction group. NO in inflammatory cells led to mucosal oxidative stress and damage[40,38]. A study also reported that the ulcer group had increased cytotoxic NO produced by iNOS inducing harmful vascular damage and accelerating the ulcer formation[41]. Additionally, TNF-α shares in this mechanism by causing gastrin overexpression stimulating iNOS production[42].

The pH results of ulcer diseased (IIa) and recovery (IIb) subgroups revealed significant decrease when compared to other groups. This is going with previous studies which demonstrated that NSAIDs free radicals' formation and PGs inhibition are the cause of pH decrease. Also, NSAIDs interfered with COX pathway which led to prostanoids production, and this interfered with the effectiveness of mucous-bicarbonate barrier which is a layer of mucous gel, bicarbonate anions and surfactant phospholipids covering the surface mucosa[43]. It was observed that the excess acid led to excess active pepsin. These two factors exacerbate the NSAIDs effects and interrupt wound healing[44].

The mean serum AST levels are considered as an early indicator of liver damage and were significantly increased in ulcer groups. The drug toxicity is the most common cause of acute liver and kidney failure[45]. It was reported that 150 mg/kg of aspirin for 28 days induced liver toxicity with dramatic rise in AST and ALT levels and hepatic cell oxidative stress led to hepatic damage which changed membrane permeability and disrupted hepatocyte transport function. These effects triggered enzyme leakage from the cells elevating their blood levels[46].

Microscopically apoptosis with pyknotic nuclei were seen in all epithelial cells reducing the thickness and widening the neck and pits. The tissue edema due to blood flow standstill and clogged vasculature caused vacuolations and separations in lamina propria and submucosa in histological results of ulcer groups (GpIIa, GpIIb).

The histological findings were in line with the information provided by a study that showed that ASA caused lysis phospholipids in mucosal epithelial cells, increasing mucosal permeability. This effect released pro-inflammatory mediators and increased leucocytic infiltration predisposing to harm to the stomach mucosal cells, complications from bleeding, and ulcers[47]. A study explained that the vacuolations of oxyntic cells and dilatation of intracellular canaliculi with ASA use might be related to the disappearance of PGs' ability to inhibit oxyntic cells[19].

Furthermore, a study revealed that sloughing with desquamation were caused by an imbalance in cell-to-cell connections and a reduction in epithelial integrity. In that effort, nuclear pyknosis was identified. This depicted cells going through atrophic degeneration and apoptosis as a result of increased ROS because of low-grade inflammatory disorder[48].

It was observed that vacuolated oxyntic cells had lost most of cell organelles. whereas vacuolated peptic cells had dilatation and elongation of the rough endoplasmic reticulum. Peptic and oxyntic nuclei looked pyknotic with heterochromatin condensation, which indicated DNA fragmentation whereas edema was related to gastric damage that led to histamine release with vasodilatation and a rise in capillary permeability and interstitial fluid[49].

Lamina propria and submucosa showed inflammatory representation in the form of congestion with infiltrated inflammatory cells. In a study, there was a notable increase in leucocytes (mostly eosinophils) in the ulcer group caused by ASA[50]. This was explained by a study that added that the inflammatory cells infiltration might be because of the release of TNF-α and IL-1[7].

These changes were aggravated in recovery group that revealed intestinal metaplastic changes in some fields in the microscopic picture. This could be explained by the direct mucosal irritating effect of ASA leaving the mucosa inflamed and destructed without treatment given to the rats promoting epithelial replacement with intestinal epithelium. It was stated that the normal gastric mucosa can progress through transitional stages including atrophic gastritis (AG) and gastric intestinal metaplasia (IM)[51]. ASA can lead to AG with gastric gland atrophy which may progress to intestinal metaplasia due to mucosal damage where ASA interacted with mucous or membrane phospholipids and can also uncouple mitochondrial oxidative phosphorylation. Alcohol and sodium salicylate induced these changes through gastric induced apoptosis recruiting TNF-α, Interleukins the one beta, and IL-6 altering the epithelial cells and activating proto-oncogenes signaling (like Bax), thus disturbing cell proliferation and apoptosis balance[52].

The direct cytotoxicity of ASA may have destroyed the surface mucous and mucous neck cells leading to focal loss of PAS-positive (+ve) reaction. The recovery revealed patterns of lost reaction in the upper part with positive reaction toward the base suggesting that the glandular epithelium was markedly diminished to the extent that mucous secreting cells may have replaced oxyntic cells loss. This was confirmed by significant reduction in the mean area percentage (%) of PAS +ve reactivity in the ulcer (IIa, IIb) groups related to all other groups. This was in line with a study which linked the ASA cytotoxicity on mucin production and barrier dysfunction to the invasion of inflammation and the oxidative distress[53]. A study interpreted that ASA group decreased PAS mean area % by increasing serum TNF-α and decreasing PGE2 in the gastric mucosa therefore decreasing mucus secretion[54]. It was observed that PAS +ve magenta-stained cells forming mucin may replace peptic and parietal cells (a process called pseudo pyloric metaplasia) in the fundic glands. Thus, PAS reaction can be seen in the base of the gland in the present work[55].

The ASA recovery group showed IM. It was recorded that AB/PAS could diagnose IM with a sensitivity better than routine H&E[56]. The gastric IM stains purple to blue with AB and -ve for PAS using AB/PAS stain[57]. A study added that all the suspected gastric lesions detected in H&E were easily diagnosed as IM with AB/PAS and authors recommended routinely performing AB/PAS to detect intestinal metaplasia in all gastric biopsies[58].

The mean area % of COX-2 & NFκB increased significantly in ulcer groups (IIa, IIb), which was consistent with a study which reported high COX-2 contents with low PGE2 in ulcer group[59]. Meanwhile, another study referred the COX-2 overexpression in gastric disorders to increased polymorphonuclear cells infiltration[60]. It was added that COX-2 can be also induced by growth factors, TNF-α in addition to NFκB[42].

It was outlined that the inhibitor (IκB) is bound to NFκB in the cytoplasm and regulate NFκB. Upon activation, proteasomal degradation of IκB released NFκB which translocated to the nucleus as p50/p65 heterodimer binding to κB site of the target genes activating their transcription. The NFκB is activated by TNF-α and IL-1[61].

In OMZ subgroup, the mean serum TNF-α and MDA were significantly improved in OMZ therapeutic group (IIIa) compared to ulcer group suggesting that OMZ may have antioxidant ability. Our results agree with a study observed that OMZ decreased HCl which could increase antioxidant enzymes but OMZ was associated with risks of gastric acid inhibition as infection liability[33].

A study mentioned that OMZ, as a PPI, acted by decreasing the aggressive factor HCl but not interfering with the protective ones[62]. In another study, it was found that OMZ treated group improved TNF-α significantly. This improvement can only be explained by the indirect decrease in oxidative stress and inflammation by the OMZ induced marked alkalinity[50].

The gastric NO in OMZ therapeutic group (IIIa) showed improvement versus ulcer group which suggests that OMZ

participated in the healing process. It was reported that constitutive NO was involved in the vagal reflex (including tissue regeneration). In addition, NO can regulate the tight junctions of the gastric and intestinal epithelial cells through inhibition of protein and lipid oxidation[63].

The OMZ control subgroup Ib and OMZ therapeutic group aggressively increased gastric juice pH when compared to the other groups. This pH reversal contributes to the OMZ healing mucosal effect. In line with our study, It was observed that OMZ treatment decreased gastric acidity[54]. This could be explained by a study which demonstrated that OMZ, as a PPI, inhibited gastric acid secretion and the pepsin activity giving time for some repair of the gastric mucosa[64].

Lastly, a significant increase in serum AST was detected in the OMZ therapeutic subgroup when compared to control subgroups which denote mild hepatic affection. This could be related to the OMZ hepatic metabolism. A study outlined that OMZ has some hepatotoxic effects on the long-term usage modifying AST and ALT[65].

Thus, new therapy is needed instead of current OMZ therapy that was reported for its nephrotoxicity, anemia dysbiosis, and malabsorption[63]. Despite the therapeutic effect of PPI and H2 receptor blockers, these medications just lower the release of acid without preventing direct damage nor promoting mucosal recovery from injury. Hence, the requirement for a multi-target medication capable of treating stomach mucosal damage[66].

Microscopically the epithelial thickness of OMZ therapeutic subgroup revealed surface epithelium regeneration where many mucous cells, oxyntic and peptic cells were normal. There were oedema and inflammatory infiltrations in lamina propria. This agreed with a study which showed a decrease in gastric damage score and epithelial desquamation in the OMZ therapeutic group through suppression of gastric acid irritating effect on the gastric mucosa[47].

The mean area % of PAS revealed a significant decrease in OMZ control subgroup suggesting that OMZ slightly affected the mucous cells. The mean PAS area % and mucin showed a statistical increase in OMZ therapeutic when related to ulcer group. In agreement with a previous study, OMZ improved ulcer index, histological features and PAS mean area %. Meanwhile there were moderate inflammatory cell infiltrations in the same study. The mucous neck cells showed sloughing which could explain the apparent widening of the gland middle part that could be observed in the OMZ therapeutic group[54].

Besides, the dilated gland neck and cellular appearance in the OMZ group may be explained by a study which pointed out that OMZ caused stomach epithelial cells genotoxicity and mutagenicity regardless the dose. The authors stated that OMZ caused parietal cell hyperplasia and canaliculi dilatation in the fundus, body and antrum of the stomach[67]. Additionally, a separate study was done on patients receiving OMZ treatment revealed that the gastric epithelial cells showed DNA oxidative stress, and chromosome breaks. The OMZ and its metabolites (as sulfone, sulfite and hydroxyomeprazole) generated nuclear pyknosis, karyorrhexis and apoptosis in stomach epithelial cells. There were nuclear defragmentation then nuclear disintegration (karyolysis), observed in patients receiving OMZ therapy both with and without gastritis[65].

A moderate +ve COX-2 immunostaining was found in OMZ therapeutic group. This was coupled to +ve cytoplasmic and nuclear NFκB immunostaining. The mean area % of COX-2 and NFκB showed significant decrease when comparing OMZ therapeutic group (IIIa) to ulcer group. In agreement with our results, A study denoted that the OMZ elevated COX-2 as a part of its anti-ulcer effect[68]. In addition, OMZ therapy against ASA led to a slight decrease in NFκB expression since OMZ, as a PPI did not directly contribute to resolution[50].

In S costus group, the mean serum MDA and TNF-α revealed a significant improvement in therapeutic subgroup (IIIb) versus ulcer group. No significance was found between S costus therapeutic subgroup and control group. Meanwhile TNF-α results showed significant decrease in S costus versus the OMZ therapeutic groups. This suggested that S costus had antioxidant and anti-inflammatory effects.

Accordingly, it was suggested that the flavonoid compounds in ulcer treatment improved MDA levels[69]. The costus root extract components (mainly flavonoids, anthraquinone, and terpenes) lowered the lipid peroxidation and modified the antioxidant defense markers[70].

Another study was conducted and added that the ethanolic S costus extract screened and revealed flavonoids (as quercetin), terpenoids, saponin, glycosides, sterols, tannins, and phenols. The flavonoids regulated apoptosis and lowered inflammation[71].

In a study, it was proved that santamarin of S costus suppressed cytotoxic NO, and TNF-α production through inhibition of NFκB translocation in macrophages[72]. Also, it has been clarified that while alkaloids, glycosides, and flavonoids in the plant extract diminished neutrophil infiltration, they also inhibited TNF-α[73].

The gastric NO improved in S costus therapeutic versus ulcer group. Unexpectedly the S costus therapeutic group and control group showed no significant difference. This suggested that S costus inhibited the cytotoxic NO. Our study was in accordance with other studies, one of which used costunolide (Co) as a treatment and showed inhibited NO production and blocked the activated NFκB[74]. The linoleic acid (a component of S costus) can be converted to γ-linolenic acid which is a PGs precursor to protect gastric mucosa[75]. In addition, alkaloids might activate COX enzyme based on another study[76].

As regards gastric pH, it was improved in S costus therapeutic group versus ulcer group. Insignificance was found between S costus therapeutic and control

group, with significant improvement in comparison with OMZ therapeutic group. A study showed that Co treated groups increased pH by suppressing the acid and NFκB and subsequently inhibiting COX-2, TNF-α and IL-1β in treated rats[74]. Concomitantly, NO may inhibit the gastric acid, elevating the pH by increasing parietal cells cGMP levels. The cGMP-activated protein kinase G will in turn decreased the H+/ K+ ATPase pump activity[77].

In addition, other authors found that flavonol glycosides (as quercetin) and terpene lactones ameliorated gastric lesions, decreased ulcer score, MDA, NO, TNF-α, COX-2 expression (via NFκB suppression) and raised gastric pH[39].

The mean serum AST levels were improved in the S costus therapeutic (IIIb) versus ulcer group with significant difference between S costus and OMZ. Interestingly, there was no significance between S costus therapeutic subgroup (IIIb) versus the control group. In line with our study, authors mentioned that Co could improve hepatic affection and might be therapeutic for liver injury[72]. It was found that flavonoids can suppress cadmium fluoride-induced oxidative stress in liver[47]. S costus could significantly inhibit the serum transaminase high levels in a dose-dependent manner in cases of induced hepatitis in mice[78].

It was confirmed that up to a dosage level of 2000 mg/kg body weight in rats, the ethanolic root extract of S costus did not cause any toxicity, hence the medication was deemed safe for additional pharmacological assessment[71].

The S costus therapeutic group showed apparently normal surface mucous, mucous neck, oxyntic and peptic cells with improvement of epithelial thickness, middle part of the gland and mucous film. Accordingly, the flavonoids antioxidant effect may lead to minimal desquamation of epithelial cells with mild gastric erosions in the treatment groups[47]. It was noted that cynaropicrin might inhibit lymphocytes[70]. It has been reported that antioxidant flavonoids, anthraquinone, and many terpenes including alpha- and beta-amyrin in S costus inhibited NFκB activation[79].

A significant rise in average area % of PAS and gastric mucin was seen in the S costus therapeutic group versus ulcer group. Compared to OMZ a significant rise was found in mucin in S costus versus OMZ therapeutic group. Interestingly no significant difference was detected in mucin in S costus therapeutic group versus control group. This suggests the repairing effect of S costus on the mucous which appeared to surpass OMZ effect.

Some authors suggested that extracted flavonoids and saponins were responsible for gastric mucous production[80]. The saponins and triterpenoids may enhance the mucous film, while tannins may have an astringent property through forming a protective layer preserving the mucous film[76].

The mean area % of COX-2 and NFκB that showed a significant decrease in S costus therapeutic versus ulcer group. Interestingly, there was a significant decrease in S costus versus OMZ therapeutic group. Concomitantly, the S costus total phenolic content may be responsible for the ability to inhibit COX-2 macrophages expression[78].

In a study, the authors linked gastric protection and ulcer inhibition to linoleic acid ability to overexpress IκB, decrease NFκB and COX-2 overexpression[75]. The polyphenols used prior to ulcer reduced COX-2 expression better than OMZ and explained it by blocking NFκB activity[81]. It was claimed that the flavonoids were able to reduce COX-2 and iNOS[82]. Thus, both could inhibit TNF-α and IL-6. The reduced TNF-α inhibited actin assembly, mitogen-activated protein kinase phosphorylation activation, and eventually NFκB transport in epithelial cells which reduce NFκB induced COX-2 transcription[83].

Some authors assumed that the pretreatment with flavonoids suppressed NFκB activation and that raised antioxidants and PGE2 expression. The flavonoids blocked IκB kinase which inhibited NFκB and so COX-2 expression which causes ulceration[84]. It has been noted that sesquiterpene lactone of S costus roots, suppressed NFκB activation and mitogen-activated protein kinase phosphorylation, whereas Santamarin component inhibited NFκB translocation in macrophages[72].

It can be concluded that aspirin induced fundic ulcers that progressed to degenerative changes and metaplasia overtime. S costus when compared to OMZ proved to be not inferior to OMZ in ameliorating ASA induced ulcers. The antioxidative and anti-inflammatory properties of S costus may be the means of this defense which were confirmed by histological, immunohistochemical, morphometric and biochemical methods.

## CONFLICT OF INTERESTS

There are no conflicts of interest.

## REFERENCES

1. Lanas A, Chan FKL (2017): Peptic ulcer disease. Lancet; 390(10094): 613-624. DOI: 10.1016/S0140-6736(16)32404-7
2. Khoder G, Al Menhali AA, Al Yassir F, Karam SM (2016): Potential role of probiotics in the management of gastric ulcer. Experimental and therapeutic medicine; 12(1): 3-17. DOI: 10.3892/etm.2016.3293
3. Chung KT, Shelat VG (2017): Perforated peptic ulcer - an update. World J Gastrointest Surg; 9(1): 1-12. DOI: 10.4240/wjgs.v9.i1.1
4. Kavitt RT, Lipowska AM, Anyane-Yeboa A, Gralnek IM (2019): Diagnosis and treatment of peptic ulcer disease. Am J Med; 132(4): 447-456. DOI: 10.1016/j.amjmed.2018.12.009
5. Galura GM, Chavez LO, Robles A, McCallum R (2019): Gastroduodenal Injury 2019: Role of Protective Factors. Curr. Gastroenterol; 21(8): 1-7. DOI: 10.1007/s11894-019-0701-x

6. Serafim C, Araruna ME, Júnior EA, Diniz M, Hiruma-Lima C, Batista L (2020): A review of the role of flavonoids in peptic ulcer (2010–2020). Molecules; 25(22): 5431-5462. DOI: 10.3390/molecules25225431

7. Mahmoud YI, Abd El-Ghffar EA (2019): Spirulina ameliorates aspirin-induced gastric ulcer in albino mice by alleviating oxidative stress and inflammation. Biomedicine & Pharmacotherapy; 109: 314-321. DOI: 10.1016/j.biopha.2018.10.118

8. Søreide K, Thorsen K, Harrison EM, Bingener J, Møller MH, Ohene-Yeboah M, Søreide JA (2015): Perforated peptic ulcer. The Lancet; 386(10000): 1288-1298. DOI: 10.1016/S0140-6736(15)00276-7

9. Lundell L (2015): The physiological background behind and course of development of the first proton pump inhibitor. Scand J Gastroenterol; 50: 680-684. DOI: 10.3109/00365521.2015.1013981

10. Pinheiro LC, Oliveira-Paula GH, Portella RL, Guimarães DA, de Angelis CD, Tanus-Santos JE (2016): Omeprazole impairs vascular redox biology and causes xanthine oxidoreductase-mediated endothelial dysfunction. Redox biology; 9: 134-143. DOI: 10.1016/j.redox.2016.08.001

11. Kostrzewska M, Świdnicka-Siergiejko A, Olszańska D, Jurkowska G, Garley M, Ratajczak-Wrona W, Jabłońska E, Jamiołkowski J, Dabrowski A (2017): The effect of omeprazole treatment on the gut microflora and neutrophil function. Clinics and research in hepatology and gastroenterology; 41(5): 575-584. DOI: 10.1016/j.clinre.2017.01.004

12. Bush J, Van Den Boom R, Franklin S (2017): Comparison of aloe vera and omeprazole in the treatment of equine gastric ulcer syndrome. Equine vet j; 50(1): 34-40. DOI: 10.1111/evj.12706

13. Forgerini M, Mieli S, Mastroianni PD (2018): Safety assessment of omeprazole use: a review. Sao Paulo Medical Journal; 136: 557-570. DOI: 10.1590/1516-3180.2018.0019220318

14. Pradeesh S, Swapna TJ (2015): In *vitro* Hepatoprotective activity of a wild medicinal plant from Western Ghats-Bidens Biternata (lour.) Merr. & Sheriff. Int J Res Rev Pharm Appl Sci, 5(2): 1246-1250. DOI: 10.62029/jmaps.v39i1.pradeesh

15. Singh R, Chahal KK, Singla N (2017): Chemical composition and pharmacological activities of Saussurea lappa: a review. J Pharmacogn Phytochem; 6(4): 1298-1308. DOI: 10.2174/22150838otkygmzmqtcvy

16. Nadda RK, Ali A, Goyal RC, Khosla PK, Goyal R (2020): Aucklandia costus (syn. Saussurea costus): Ethnopharmacology of an endangered medicinal plant of the Himalayan region. J Ethnopharmacol; 263: 113199-113216. DOI:10.1016/j.jep.2020.113199

17. Gautam H, Asrani RK (2018): Phytochemical and pharmacological review of an ethnomedicinal plant: Saussurea lappa. Vet Res Int; 6(1): 1-9. DOI: 10.1016/j.vetRI.2018.11.145

18. Deabes M, Aboulthana W, Marzouk EE, Mohamed MI, Ahmed KA (2021): Evaluation of hepato-and neuroprotective effect of chemical constituents in Saussurea costus extract against the toxicity induced by chloropyrofos ethyl in rats. Egyptian Journal of Chemistry; 64(2): 631-647. DOI: 10.21608/EJCHEM.2021.55840.3199

19. Abd-Elhamid A, Salem MA, Mahmoud SM (2021): Histological Study of the Caffeine Effect on Intact Gastric Mucosa and Induced Gastric Ulcer in Adult Male Albino Rats. EJH; 44(1): 163-187. DOI: 10.21608/ejh.2020.29657.1285

20. Torres OJ, SANTOS OJ, MOURA RS, Serra HO, Ramos VP, MELO SP, Loureiro CM (2018): Activity of orbignya phalerata and euterpe edules in the prevention and treatment of peptic ulcer in rats. Arq Bras Cir Dig; 31(3): e1390-1393. DOI: 10.1590/0102-672020180001e1390

21. Zatroch KK, Knight CG, Reimer JN, Pang DS (2016): Refinement of intraperitoneal injection of sodium pentobarbital for euthanasia in laboratory rats (Rattus norvegicus). BMC veterinary research; 13(1): 1-7. DOI: 10.1186/s12917-017-0982-y

22. Fathy SM, Drees EA (2015): Protective effects of Egyptian cloudy apple juice and apple peel extract on lipid peroxidation, antioxidant enzymes and inflammatory status in diabetic rat pancreas. BMC complementary and alternative medicine; 16(1): 1-4. DOI: 10.1186/s12906-015-0957-0

23. Khattab HA, Aljehany BM (2020): Costus afer (Costaceae, Zingiberales) leaf extract ameliorates naproxen-induced gastric ulcer in rats. Trop J Pharm Res;19(12): 2615-2622. DOI: 10.4314/tjpr.v20i5.31

24. Suvarna KS, Layton C, Bancroft JD (2018): Bancroft's Theory and Practice of Histological Techniques. 8th edition, E-Book, Elsevier Health Sciences: 337-362. DOI: 10.1016/b978-0-7020-6864-5.05001-5

25. Makav M, Gelen V, Gedikli S, Atila Uslu G, Uslu H, Eroğlu H (2020): Therapeutic effect of Tarantula cubensis extract on indomethacin induced gastric ulcers in rats. Thai Journal of Veterinary Medicine; 50(4): 559-566. DOI: 10.56808/2985-1130.3062

26. Hamad AK, Kadhem MA (2020): Prophylactic Effect of Ethanolic Extract of Saussurea Costus Roots Against Hepato-Renal Toxicity Induced by Diazinon in Chickens. Al-Anbar J Vet Sci; 13(2): 34-51. DOI: 10.37940/AJVS.2020.13.2.4

27. Miranda KM, Espey MG, Wink DA (2001): A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric oxide; 5(1):62-71. DOI: 10.1006/niox.2000.0319

28. Emsley R, Dunn G, White IR (2010): Mediation and moderation of treatment effects in randomized controlled trials of complex interventions. Start Methods Med Res; 19(3): 237-270. DOI: /10.1177/0962280209105014

29. Xie X, Ren K, Zhou Z, Dang C, Zhang H (2022): The global, regional and national burden of peptic ulcer disease from 1990 to 2019: a population-based study. BMC gastroenterology; 22(1): 58-70. DOI: 10.1186/s12876-022-02130-2

30. Jin Y, Zhang M, Wang Y, Lu Y, Liu T, Yang G, Song S, Liu W (2022): Protective effect and potential mechanism of the traditional chinese medicine shaoyao-gancao decoction on ethanol-induced gastric ulcers in rats. Evidence-Based Complementary and Alternative Medicine; 2022: 1-16. DOI: 10.1155/2022/3069089

31. Song H, Hou X, Zeng M, Chen X, Chen X, Yang T, Xu F, Peng J, Peng Q, Cai X, Yu R (2020): Traditional Chinese Medicine Li-Zhong-Tang accelerates the healing of indomethacin-induced gastric ulcers in rats by affecting TLR-2/MyD88 signaling pathway. J Ethnopharmacol; 259: 112979-112993. DOI: 10.1016/j.jep.2020.112979

32. Aziz RS, Siddiqua A, Shahzad M, Shabbir A, Naseem N (2019): Oxyresveratrol ameliorates ethanol-induced gastric ulcer via downregulation of IL-6, TNF-α, NF-κB and COX-2 levels, and upregulation of TFF-2 levels. Biomedicine & Pharmacotherapy; (110): 554-560. DOI: 10.1016/j.biopha.2018.12.002

33. Ruiz-Hurtado PA, Garduño-Siciliano L, Dominguez-Verano P, Martinez-Galero E, Canales-Martinez MM, Rodriguez-Monroy MA (2021): Evaluation of the gastroprotective effects of Chihuahua propolis on indomethacin-induced gastric ulcers in mouse. Biomedicine & Pharmacotherapy;137: 111345-111359. DOI: 10.1016/j.biopha.2021.111345

34. Soleimanzadeh A, Talavi N, Yourdshahi VS, Bucak MN (2020): Caffeic acid improves microscopic sperm parameters and antioxidant status of buffalo (Bubalus bubalis) bull semen following freeze-thawing process. Cryobiology; 95: 29-35. DOI: 10.1016/j.cryobiol.2020.06.010

35. Zhang W, Lian Y, Li Q, Sun L, Chen R, Lai X, Lai Z, Yuan E, Sun S (2020): Preventative and therapeutic potential of flavonoids in peptic ulcers. Molecules; 25(20): 4626-4656. DOI: 10.3390/molecules25204626

36. Kamar SS, Latif NS, Elrefai MF, Amin SN (2020): Gastroprotective effects of nebivolol and simvastatin against cold restraint stress-induced gastric ulcer in rats. Anatomy & Cell Biology; 53(3): 301-312. DOI: 10.5115/acb.20.055

37. Badriyya E, Mandasari Z, Aldi Y, Almahdy A (2021): Gastroprotective Effect of Propolis Against Male White Mice Gastric Ulcers Induced by Aspirin. In2nd International Conference on Contemporary Science and Clinical Pharmacy 2021 (ICCSCP 2021). Advances in Health Sciences Research, Atlantis Press, 40: 98-103. DOI: 10.2991/ahsr.k.211105.014

38. Gemici B, Birsen İ, İzgüt-Uysal VN (2023): The apelin-apela receptor APJ is necessary for formation and healing of ischemia reperfusion-induced gastric ulcer in rats. Peptides; 166: 171027-171035. DOI: 10.1016/j.peptides.2023.171027

39. Abd-Eldayem AM, Alnasser SM, Abd-Elhafeez HH, Soliman SA, Abdel-Emam RA (2022): Therapeutic versus preventative use of Ginkgo biloba Extract (EGb 761) against indomethacin-induced gastric ulcer in mice. Molecules; 27(17):5598-5618. DOI: 10.3390/molecules27175598

40. Akmal MN, Abdel Aziz I, Nur Azlina MF (2023): Piper sarmentosum Roxb. methanolic extract prevents stress-induced gastric ulcer by modulating oxidative stress and inflammation. Front Pharmacol; 13: 971443-971453. DOI: 10.3389/fphar.2022.971443

41. Liu R, Zhu N, Hao Y, Liu X, Kang J, Mao R, Yu X, Li Y (2023): The Protective Effect of Walnut Oligopeptides against Indomethacin-Induced Gastric Ulcer in Rats. Nutrients. Mar 30;15(7): 1675-1693. DOI: 10.3390/nu15071675

42. Mahmoud MF, Abdo W, Nabil M, Drissi B, El-Shazly AM, Abdelfattah MA, Sobeh M (2023): Apple (Malus domestica Borkh) leaves attenuate indomethacin-induced gastric ulcer in rats. Biomedicine & Pharmacotherapy; 160: 114331-114339. DOI: 10.1016/j.biopha.2023.114331

43. Mugo NW (2022): Chapter Five: Discussion, MSc. Anti-Ulcerogenic Activity of Leaf Extract of Capparis Cartilaginea Decne on Ethanol and Indomethacin-Induced Peptic Ulcers in Wistar Rats (Doctoral dissertation, JKUAT-COHES): 35-37. http://ir.jkuat.ac.ke/handle/123456789/5792 DOI: 10.18203/2320-6012.ijrms20204213

44. Beiranvand M (2022): A review of the most common in *vivo* models of stomach ulcers and natural and synthetic anti-ulcer compounds a comparative systematic study. Phytomedicine Plus; 2(2): 100264-100288. DOI: 10.1016/j.phyplu.2022.100264

45. Chen S, Zhang CL, Zhou XF, Gao Y, Chen H, Fu BD, Chen H, Fu BD, Yi PF, Shen HQ (2020): Antiinflammatory and antioxidative properties of helicid protect against CCl4 induced acute liver injury in mice. Biotech Histochemist; 95(7): 483-489. DOI: 10.1080/10520295.2020.1718210

46. Amin HM, Youssef MA (2020): Immunological, hematological and biochemical effects of aspirin in low and high doses in male albino rats. Eur J Mol Clin Med; 7(11): 6700-6713. DOI: 10.1016/j.toxrep.2022.03.010

47. Busman H, Utama WT, Hanriko R, Amper JM, Indriyani I (2022): Effect of Ajwa Date (Phoenix dactilyfera L.) extract in aspirin induced peptic ulcer model. Health Biotechnology and Biopharma (HBB); 6(3): 23-32. DOI: 10.22034/HBB.2022.21

48. Radwan DM, Ebrahim NA, Farag EA, Sofi MA (2019): Histological Study on the Possible Protective Effect of Co-enzyme Q10, Evening Primrose and Esomeprazole on Indomethacin Induced Gastric Ulcer in Adult Male Albino Rats. Nat Sci; 17(12): 220-240. DOI:10.1007/s11033-019-04933-1

49. Ismail OI, El-Meligy MMS (2022): Curcumin ameliorated low dose-Bisphenol A induced gastric toxicity in adult albino rats. Sci Rep; 12(1): 10201-10215. DOI: 10.1038/s41598-022-14158-1

50. Ibrahim MF, Allam FA (2022): Potential stem cell—conditioned medium and their derived exosomes versus omeprazole in treatment of experimental model of gastric ulcer. Acta Histochemica; 124(4): 151896-151910. DOI:10.1016/j.acthis.2022.151896

51. Ahmad AI, Lee A, Caplan C, Wikholm C, Pothoulakis I, Almothafer Z, Raval N, Marshall S, Mishra A, Hodgins N, Kang IG, Chang RK, Dailey Z, Daneshmand A, Kapadia A, Oh JH, Rodriguez B, Sehgal A, Sweeney M, Swisher CB, Childers DF, O'Connor C, Sequeira LM, Cho W (2022): Gastric intestinal metaplasia development in African American predominant United States population. World J Gastrointest Endosc; 14(10): 597-607. DOI: 10.4253/wjge.v14.i10.597

52. Han L, Li T, Wang Y, Lai W, Zhou H, Niu Z, Su J, Lv G, Zhang G, Gao J, Huang J (2023): Weierning, a Chinese patent medicine, improves chronic atrophic gastritis with intestinal metaplasia. J Ethnopharmacol; 309: 116345-116358. DOI: 10.1016/j.jep.2023.116345

53. Elwan WM, Ibrahim MAA (2019): Effect of tartrazine on gastric mucosa and the possible role of recovery with or without riboflavin in the adult male albino rat. EJH; 42 (2): 297-311. DOI: 10.21608/ejh.2019.6312.1043

54. El-Kerdasy HI, Mousa HR (2020): A Comparative Study Between The Therapeutic Role of Adipose Derived Mesenchymal Stem Cells and Omeprazole in Regeneration of Gastric Ulcer Induced by Aspirin in Albino Rats: Histological and Immunohistochemical Study. Egyptian Journal of Histology (EJH); 44(4): 993-1006. DOI: 10.21608/ejh.2020.28223.1277

55. Livzan MA, Mozgovoi SI, Gaus OV, Shimanskaya AG, Kononov AV (2023): Histopathological Evaluation of Gastric Mucosal Atrophy for Predicting Gastric Cancer Risk: Problems and Solutions. Diagnostics; 13(15): 2478 -2492. DOI: 10.3390/diagnostics13152478

56. Alkhamiss AS (2020): Evaluation of Better Staining Method among Hematoxylin and Eosin, Giemsa and Periodic Acid Schiff-Alcian Blue for the Detection of Helicobacter pylori in Gastric Biopsies. Malays J Med Sci; 27(5): 53-61. DOI: 10.21315/mjms2020.27.5.6

57. Yin J, Yi J, Yang C, Xu B, Lin J, Hu H, Wu X, Shi H, Fei X (2021): Chronic atrophic gastritis and intestinal metaplasia induced by high salt and N methyl N' nitro N nitrosoguanidine intake in rats. Exp Ther Med; 21(4): 315. DOI: 10.3892/etm.2021.9746

58. Kiliçarslan A, Varli G (2023): Should Special Staining (AB/PAS) be Routinely Performed in Gastric Biopsies for the Detection of Intestinal Metaplasia?. International Journal of Surgical Pathology:1-6. DOI: 10.1177/10668969231169050

59. Qiujian Y, Zongcai F, Liping H, Jingwei H, Zhongliu Z, Fang L (2021): Ellagic acid (EA), a tannin was isolated from Eucalyptus citriodora leaves and its anti-inflammatory activity. Medicinal Chemistry Research; 30: 2277-2288. DOI: 10.1007/s00044-021-02806-6

60. Mendivil EJ, Sandoval-Rodriguez A, Meza-Ríos A, Zuñiga-Ramos L, Dominguez-Rosales A, Vazquez-Del Mercado M, Sanchez-Orozco L, Santos-Garcia A, Armendariz-Borunda J (2019): Capsaicin induces a protective effect on gastric mucosa along with decreased expression of inflammatory molecules in a gastritis model. Journal of Functional Foods; 359:3345-351. DOI: 10.1016/j.jff.2019.06.002

61. Khongthong P, Roseweir AK, Edwards J (2019): The NF-KB pathway and endocrine therapy resistance in breast cancer. Endocrine-related cancer; 26(6): R369-R380. DOI: 10.1530/ERC-19-0087

62. Boeing T, de Souza J, da Silva RD, Mariano LN, da Silva LM, Gerhardt GM, Cretton S, Klein-Junior LC, de Souza P (2023): Gastroprotective effect of Artemisia absinthium L.: A medicinal plant used in the treatment of digestive disorders. J Ethnopharmacol; 312: 116488-116496. DOI: 10.1016/j.jep.2023.116488

63. Pineda-Peña EA, Chávez-Piña AE (2023): Gaseous mediators (H2S, NO and CO) a new approach in gastrointestinal protection. MJ Medical Research ICSa; 11(21): 39-47. DOI: 10.29057/mjmr.v11i21.9436

64. Wang X, Fu S, Zhang C, Nie X, Liao W, Zhao M, Liu F (2021): Gastroprotective effect of ethanol extracts from bark of magnolia officinalis on ethanol-induced gastric mucosal damage in rats. BioMed Research International; 2021: 1-13. DOI: 10.1155/2021/6688414

65. da Mata AM, Paz MF, de Menezes AA, Dos Reis AC, da Silva Souza B, de Carvalho Sousa CD, Machado SA, Medeiros TS, Sarkar C, Islam MT, Sharifi-Rad J (2022): Evaluation of mutagenesis, necrosis and apoptosis induced by omeprazole in stomach cells of patients with gastritis. Cancer Cell Int; 22(1): 154-167. DOI: 10.1186/s12935-022-02563-5

66. Chen T, Chen J, Bao SC, Zhang JX, Wei HL, Zhou XY, Hu X, Liang Y, Li JT, Yan SG (2024): Mechanism of Xiaojianzhong decoction in alleviating aspirin-induced gastric mucosal injury revealed by transcriptomics and metabolomics. J Ethnopharmacol; 318(PartA): 116910-116925. DOI: 10.1016/j.jep.2023.116910

67. Braga AL, do Nascimento PB, Paz MF, de Lima RM, Santos JV, de Alencar MV, de Meneses AA, Junior AL, Islam MT, Sousa JM, Melo-Cavalcante AA (2021): Antioxidative defense against omeprazole-induced toxicogenetical effects in Swiss mice. Pharmacological Reports; 73: 551-562. DOI: 10.1007/s43440-021-00219-1

68. Lien HM, Wang YY, Huang MZ, Wu HY, Huang CL, Chen CC, Hung SW, Chen CC, Chiu CH, Lai CH (2022): Gastroprotective Effect of Anisomeles indica on Aspirin-Induced Gastric Ulcer in Mice. Antioxidants; 11(12): 2327-2339. DOI: 10.3390/antiox11122327

69. Kim YS, Lee JH, Song J, Kim H (2020): Gastroprotective effects of inulae flos on HCl/Ethanol-Induced gastric ulcers in rats. Molecules; 25(23): 5623-5635. DOI:10.3390/molecules25235623

70. Ashry M, Galal ELSahra D, Abdel-Wahhab KG, Abdelsalam ME, Mourad HH, El-Bitar AM, Gomaa HF (2022): Saussurea Costus Extract Has Anti-inflammatory, Antioxidant, and Hormonal Effects Against Testicular Toxicity Induced by Oxaliplatin in Male Albino Rats. Iran J Toxicol; 16(2): 83-90. DOI: 10.32598/IJT.16.2.894.1

71. Kumar JV, Pratap CG (2023): assessment of the anti-obesity efficacy of ethanolic extracts of saussurea lappa (costus) roots in cafeteria diet fed obese rats. Eur Chem Bull; 12(5): 1390-1402. DOI: 10.48047/ecb/2023.12.si5a.015

72. Liu XN, Li HM, Wang SP, Zhang JZ, Liu DL (2021): Sesquiterpene lactones of Aucklandia lappa: Pharmacology, pharmacokinetics, toxicity, and structure–activity relationship. Chin Herb Med; 13(2): 167-176. DOI: 10.1016/j.chmed.2020.11.005

73. Geetha KM, Anitha KN (2022): Protective Effect of Nigella sativa Seeds Extract against Ethanol-Induced Gastric Ulcer in Rats: Possible Soluble Epoxide Hydrolase Inhibition. Indian J Pharm Sci.; 84 (1): 41-48. DOI:10.36468/pharmaceutical-sciences.893

74. Nan L, Nam HH, Choo BK (2020): Costunolide inhibits inflammation in LPS-induced RAW264. 7 cells and ameliorates gastric acid reflux-induced esophageal injury in rat model. Appl Biol Chem; 63(1): 33-41. DOI: 10.1186/s13765-020-00514-0

75. Long X, Zhao X, Wang W, Zhang Y, Wang H, Liu X, Suo H (2019): Protective effect of silk worm pupa oil on hydrochloric acid/ethanol-induced gastric ulcers. J Sci Food Agric; 99(6): 2974-2986. DOI:10.1002/jsfa.9511

76. Ahmed O, Nedi T, Yimer EM (2022): Evaluation of anti-gastric ulcer activity of aqueous and 80% methanol leaf extracts of Urtica simensis in rats. Metabolism Open; 14: 100172-100178. DOI:10.1016/j.metop.2022.100172

77. 77. Jalil AT, Hassan MM, Ziyad RA, Jasim I, Zabibah R, Fadhil A (2023): PDE5 inhibitors and gastric mucosa: implications for the management of peptic ulcer disease. Naunyn-Schmiedeberg's Arch Pharmacol; 2023: 1-7. DOI: 10.1007/s00210-023-02503-8

78. Urvashi, Kaur R (2023): Chapter 18: Saussurea Lappa C.B. Clarke KushtaKut. In: Sharma A, Nayik GA (eds) Immunity Boosting Medicinal Plants of the Western Himalayas. (eBook) Springer Nature, Singapore, Singapore: 433-462. DOI: 10.1007/978-981-19-9501-9_18

79. Fekry E, Awny M, Refaat G, Arafat H (2023): Ameliorative Role of Saussurea Lappa Root Extract. Zagazig Journal of Forensic Medicine; 21(1): 172-189. DOI: 10.21608/zjfm.2023.188310.1139

80. Putri CA, Ramadani AP, Amanati M (2021): Preventive Activity of Muntingia calabura Fruits Extract in Aspirin-induced Gastric Ulcer Rat. IJPST; 8(3): 106-110. DOI: 10.1016/j.sciaf.2023.e01672

81. Shaik RA, Eid BG (2022): Piceatannol affects gastric ulcers induced by indomethacin: association of antioxidant, anti-inflammatory, and angiogenesis mechanisms in rats. Life; 12(3): 356-369. DOI: 10.3390/life12030356

82. Ragheb AY, Masoud MA, El Shabrawy MO, Farid MM, Hegazi NM, Mohammed RS, Marzouk MM, Aboutabl ME (2023): MS/MS-based molecular networking for mapping the chemical diversity of the pulp and peel extracts from Citrus japonica Thunb.; in *vivo* evaluation of their anti-inflammatory and anti-ulcer potential. Scientific African; 20: e01672-016698. DOI: 10.1016/j.sciaf.2023.e01672

83. Yi CO, Jeon BT, Shin HJ, Jeong EA, Chang KC, Lee JE, Lee DH, Kim HJ, Kang SS, Cho GJ, Choi WS (2011): Resveratrol activates AMPK and suppresses LPS-induced NF-κB-dependent COX-2 activation in RAW 264.7 macrophage cells. Anatomy & Cell Biology; 44(3): 194-203. DOI: 10.5115/acb.2011.44.3.194

84. Park HS, Seo CS, Baek EB, Rho JH, Won YS, Kwun HJ (2021): Gastroprotective effect of myricetin on ethanol-induced acute gastric injury in rats. Evidence-Based Complementary and Alternative Medicine; 2021: 1-9. DOI: 10.1155/2021/9968112. eCollection 2021.

**الملخص العربى**

# دراسة مقارنة لتأثير التحسيني لمستخلص القسط الهندي مقابل الأوميبرازول على الغشاء المخاطي القاعدي في نموذج القرحة المعدية

**سارة عادل حسني محمد، ميرا فاروق يوسف يعقوب، الاء عصام الدين علي فصيح، أمل إلهام أحمد فارس**

**قسم علم الأنسجة الطبية، كلية الطب، جامعة القاهرة**

**الخلفية:** يعد مرض القرحة المعدية عبئا صحيا عالميا كبيرا فهو يؤدي إلى مضاعفات خطيرة إذا أهمل دون علاج. وهدفت هذه الدراسة إلى تقييم التأثير التحسيني لمستخلص القسط الهندي مقابل الأوميبرازول - باعتباره أحدث علاج مستخدم للقرحة- في القرحة المعدية المستحدثة بعقار الأسبرين.

**المنهجية:** تم تقسيم تسعة وعشرون جرذا إلى: المجموعة الضابطة )مج (I والتي تنقسم إلى ثلاث مجموعات فرعية: (مجموعة الماء المقطر، ومجموعة الأوميبرازول الضابطة التي تلقت أوميبرازول لمدة عشرة أيام، ومجموعة مستخلص القسط الضابطة التي تلقت السوسوريا مستخلص القسط لمدة عشرة أيام)، والمجموعة الثانية: المجموعة الفرعية مج IIa مجموعة القرحة المعدية المرضية، والمجموعة الفرعية مج IIb مجموعة التعافي، أما المجموعة الثالثة: المجموعة العلاجية وتنقسم إلى: مجموعة الأوميبرازول الفرعية مج IIIa ومجموعة القسط الفرعية مج IIIb . تم إعطاء الأسبرين يوميا عن طريق الفم لمدة خمس أيام لجميع المجموعات. ثم تلقت مجموعة مج IIIa عقار الأوميبرازول ، وتلقت المجموعة مج IIIb مستخلص القسط كلاهما لمدة عشرة أيام. ثم تم قياس المالوندياليديهايد (MDA) ، وعامل نخر الورم ألفا (TNF-α) ومستوى ناقلة أمين الأسبارتات (AST) في البلازما بالإضافة الى قياس أكسيد النيتريك المعدي في الغشاء المخاطي للمعدة (NO) ، والبروستاجلاندين E٢ (PGE٢) في عينات المعدة المجمدة. خضعت عينات المعدة للدراسات الآتية: النسيجية؛ والهستوكيميائة مناعية؛ والقياسات المترية الشكلية. النتائج: كشفت المجموعات الفرعية مج IIa وIIb عن فقدان ملحوظ في بطانة المعدة مع خلايا تحتوي على نوى داكنة. كان هناك زيادة كبيرة في متوسط قيم MDA وTNF-α وAST ومتوسط المساحة المئوية للظهور المناعي الإيجابي لCycloxygenase-٢ (COX٢) والعامل النووي كابا B (NFκB) مع انخفاض في متوسط المساحة ٪ من {حمض البيريودك شيف} (PAS)في المجموعة IIa و IIbمقارنة بالمجموعات الأخرى. أظهر علاج الأوميبرازول ومستخلص القسط تحسن معتبر احصائيا للتغيرات الكيميائية الحيوية والنسيجية والكيميائية المناعية المستحدثة بالأسبرين على الغشاء المخاطي للمعدة.

**الاستنتاج:** يمكن أن يؤدي الأسبرين إلى تغيرات تحولية على مستوى الخلايا في التركيب النسيجي للغشاء المخاطي للمعدة. أحدث مستخلص القسط نتائج منافسة للأوميبرازول مع تحسن كبير في الغشاء المخاطي في نموذج الجرذ لقرحة المعدة بالإضافة إلى تحسن في وظائف الكبد.