Nature and Science

Websites:
http://www.sciencepub.net/nature
http://www.sciencepub.net

Emails:
naturesciencej@gmail.com
editor@sciencepub.net

MARSLAND PRESS
Multidisciplinary Academic Journal Publisher

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

Prof. Dr. Dina Mohamed Radwan[1], Dr. Noha Abd Ellatif Ebrahim[2], Dr. Eman Abas Farag[1], Marwa Abd ElAziz Sofi[2]

[1]Histology Department, Faculty of Medicine, Cairo University, Egypt
[2]Histology Department, Faculty of Medicine, Fayoum University, Egypt
marwaabdelaziz1811@yahoo.com

Abstract: Introduction: The non-steroidal anti-inflammatory drug (NSAID) Indomethacin is widely used especially in treatment of osteoarthritis and rheumatoid arthritis. However, it has many side effects; the most common of them is gastrointestinalin tolerance and ulceration. Coenzyme Q10 (CoQ10) and Evening primrose (EP) were reported to have anti-inflammatory and antioxidant properties. **Aim of the Work:** To investigate the histopathological effects of Indomethacin on the fundic mucosa of adult male albinorats and the possible protective role of Coenzyme Q10, Evening primrose and esomeprazole. **Materials and Methods:** Forty eight adult male albino rats were randomized into 5 groups (N=8). Group I (Control). Group II (INDO): received 50 mg/kg of indomethacin dissolved in gum acaciaorally. Group III (CoQ10+INDO): received Coenzyme Q10 10mg/kg for 2 days then INDO on the 2nd day 1 hour after CoQ10. Group IV (EP+INDO): received evening primrose intraperitoneally 10g/kg for 2 days then INDO on the2nd day 1 hour after EP. Group V (ESP+INDO (: Subgroup Va (low dose): received 5mg/kg/day esomeprazole orally for 2 days then INDO on the2nd day 1 hour after ESP. Subgroup Vb (high dose): treated as group Va but received 20mg/kg/dayESP. At the end of the experiment, ulcer index and antioxidant parameters were evaluated. The fundic mucosa was processed and stained with H & E, PAS stains as well as Caspase-3 and PCNA immunostains. Morphometric measurements and statistical analysis were done. **Results:** Erosions, congestion, extravasated RBCs and inflammatory cell infiltration were detected in fundic mucosa of INDO group. In CoQ10+INDO group, EP+INDO and ESO+INDO high dose the structure of the mucosa was restored. There was marked increase in mucus secretion, significantly increased ulcer index and decreased MDA level. A significant decrease in area % of PAS reaction in INDO group was revealed as compared with the other groups. There was a significant increase in area % of Caspase immunoreaction and a significant decrease in area % of PCNA expression in INDO group as compared with other groups. **Conclusion:** Coenzyme Q10, Evening primrose and esomeprazole (high dose) can partly protect the fundic mucosa against indomethacin induced damage.
[Dina Mohamed Radwan, Noha Abd Ellatif Ebrahim, Eman Abas Farag, Marwa Abd ElAziz Sofi. **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* 2019;17(12):220-240]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 29. doi:10.7537/marsnsj171219.29.

Key words: Indomethacin, gastritis, CoQ10, evening primerose, esomeprazole, caspase, PCNA and rat.

1. Introduction

Gastric ulcer is one of the most common disorders of the gastrointestinal tract. It affects 5% of the population around the world, so its prevention and management are considered very important challenges. Researchers have revealed several causes of gastric ulcer leading to an imbalance between aggressive and intrinsic defensive factors. The aggressive factors include non-steroidal anti-inflammatory drugs (NSAIDs), alcohol, psychological stress and Helicobacter pylori infection while cytoprotective intrinsic factors include mucosal blood flow, bicarbonate, mucus, cell renewal, growth factors and prostaglandins ***(Abourehab, 2017; Escobedo-Hinojosa et al., 2018).***

Gastric damage induced by NSAIDs is the most common and dangerous side-effect of these drugs. They account for 25% of gastric ulcer cases. Indomethacin is considered to be the most common NSAIDs known to induce experimental gastric ulcer. It has been documented to have a higher potential to cause gastric injury than other commonly-used NSAIDs ***(Varga et al., 2017).***

Coenzyme Q10 (CoQ10), a peculiar lipophilic antioxidant, is an essential component of the mitochondrial electron-transport chain. It is involved in the manufacturing of adenosine triphosphate (ATP). It has been linked with improving cognitive functions. Previous studies have revealed its possible

antiulcerogenic effect on the stomach (***Zhang et al., 2018***).

Evening primrose (EP) is a biennial herb originating from Mexico and Central America. Throughout the years, it has become a widespread plant in Americas, Europe and parts of Asia. The Native Americans valued EP stems and leaf juices as to alleviate inflammation. Evening primerose has also been used in medicine as a remedy for neuralgia, skin, liver, kidney and gastrointestinal diseases (***Munir et al., 2017).***

Esomeprazole (ESP) is a proton-pump inhibitor which reduces stomach acid. It is used in the treatment of dyspepsia, peptic ulcer disease, gastro esophageal reflux disease, and Zollinger-Ellison syndrome (***Cho et al., 2019)***. Two doses of ESP were used to detect whether the effect of ESP was dose dependent or not.

Current treatment of gastric ulcer is associated with several side-effects, which emphasizes the need for new therapeutic modalities. It is believed that the therapeutic outcome of Coenzyme Q10 and Primerose can be more effective to treat gastric ulcer.

Aim of the work

The aim of this study is to investigate the possible protective effect of coenzyme Q10, evening primrose and esomeprazole on indomethacin induced gastric ulcer in adult male albino rats using histological and morphometric studies.

2. Materials and methods

Materials

Drugs:

1. Indomethacin: (Indomethacin) from Safe pharmaceutical for Pharco Pharmaceuticals in the form of soft gelatinous capsules (25 mg). Each capsule was dissolved in 5 ml gum acacia solution (each mg of gum acasia dissolved in 100 ml of water). Each ml of gum acacia solution contained 5mg of indomethacin. Each rat received 2 ml of the solution orally (indomethacin dose is 10mg/rat) **) *Sabiu et al., 2015)***.

2. Co-enzyme Q10: (Coenzyme Q10) supplied from Arab Company for Pharmaceutical and Medicinal plants MEPACO – MEDIFOOD Enshas El Raml - Sharkeya - Egypt in the form of gelatin capsules (30 mg). Each capsule was dissolved in 3 ml of olive oil. Each rat received 0.2 ml of the solution intraperitoneally **(*Ivanov et al., 2014*)**.

3. Evening primrose: (Primrose Plus) supplied from Pharma Plaza Building, Asma Fahmy st., El Nozha, Nasr City, Egypt in the form of soft gelatin capsules (1000 mg). Each soft gelatin capsule contains: Evening primerose oil 1000 mg and Alpha-tocopherol acid (GLA). Each rat received 2g intraperitoneally **(*Abo-greesha et al., 2014)***.

4. Esomeprazole: (trade name Esomelodan) supplied from EVA PHARMA. For Pharmaceutical and Medical Appliance S.A.E. Kafr El Gabal - Haram -Giza – Egypt in the form of tablets (40 mg). Each tablet contains: Esomeprazole magnesium trihydrate 44.6 mg (equivalent to esomeprazole 40mg). It was dissolved in 2 ml of distilled water. Rats of low dose group received 0.05 ml. Rats of high dose group received 0.2 ml **(*Sabiu et al., 2015)***.

Animals:

Forty eight adult male albino rats were used in this study, 10-12 weeks old, with average weight weighing (180-220) gm. The animals were locally bred at the Animal House of Faculty of Science, Fayoum University in hygienic stainless steel cages and in a clean well ventilated room. Standard laboratory chow and tap water were available *ad libitum*. The experimental design was approved by the animal ethics committee, Faculty of Science, Fayoum University.

Experimental design:

Rats were randomly divided into five groups, housed in separate cages:

●**Group I (Control group):** included eight rats, this group was further subdivided into the following equal subgroups:

Subgroup Ia: two rats without intervention.

Subgroup Ib: two rats received 0.5 ml gum acacia dissolved in distilled water (solvent of indomethacin) orally using an oral gastric tube on the 2nd day of experiment (corresponding to groups II & IV).

Subgroup Ic: two rats were injected with 0.5 ml olive oil (solvent of CoQ10) intraperitoneally and on the 2nd day received in addition 0.5 ml gum acacia as in subgroup Ib (corresponding to group III).

Subgroup Id: two rats received 0.5 ml distilled water (solvent of esomeprazole) orally using an oral gastric tube and on the 2nd day received in addition 0.5 ml gum acacia (corresponding to group V).

Rats were sacrificed at the same time of the corresponding experimental groups.

●**Group II (Indomethacin treated group) (INDO):** included eight rats which received single oral dose of 50 mg/kg of indomethacin dissolved in gum acacia on the 2nd day of experiment ***) Sabiu et al., 2015).***

●**Group III (Coenzyme Q10 and indomethacin treated group) (CoQ10 +INDO):** included eight rats which were injected intraperitoneally with coenzyme Q10 (dissolved in olive oil) in a daily dose 10mg/kg **(*Ivanov* et *al., 2014*)** for 2 consecutive days. Indomethacin was given on the 2nd day of experiment 1 hour after the CoQ10.

●**Group IV (Primrose and indomethacin treated group) (EP+INDO):** included eight rats which were injected with evening primrose intraperitoneally in a daily dose of 10g/kg **(*Abo-***

greesha et al., 2014) for 2 consecutive days. Indomethacin was given on the2nd day of experiment 1 hour after the EP.

●**Group V (Esomeprazole and indomethacin treated group):** This group was subdivided into 2 subgroups 8rats each:

Subgroup Va (low dose) (ESP (a)+INDO): eight rats received 5mg/kg/day of esomeprazole (dissolved in distilled water) **(*Sabiu et al., 2015)*** orally using a gastric tube for 2 consecutive days then, indomethacin was given on the 2nd day of experiment 1 hour after the ESP.

Subgroup Vb (high dose) (ESP (b)+INDO): eight rats received 20mg/kg/day **(*Sabiu et al., 2015)*** of esomeprazole (dissolved in distilled water) orally using a gastric tube for 2 consecutive days then, indomethacin was given on the 2nd day of experiment 1 hour after the ESP.

Ulcer induction:

Rats were deprived of food but had free access to water 24 hours before ulcer induction. Gastric ulceration was induced by giving the rats a single oral dose of indomethacin (50 mg/kg) dissolved in gum acacia on the 2nd day of experiment 1 hour after the second dose of prophylaxis. Ulceration was detected according to the method of Dawud ***et al., 2014,*** 6 hours after indomethacin administration ***(Kim et al., 2011; Abdel-Raheem and Bamagous, 2016).***

Assessment of ulcer:

After six hours of ulcer induction, the animals were anesthetized using injection of thiopental sodium 50 mg/kg subcutaneously. The abdomen was then rapidly dissected, and stomach was removed and opened along greater curvature and the gastric content was drained into a centrifuge tube then cleansed gently with normal saline. Detection of ulcers was done.

Measurement of ulcer index:

Ulcers were evaluated using eye piece of the microscope. The ulcer score for each group was calculated as the mean number of ulcers/stomach/rat in each group (Table 1). From these values, an Ulcer index (UI) was calculated by multiplying each group's ulcer score x 100. Subsequently, the net preventive index was calculated as 100% x (UI of ulcerated group - UI of treated group)/UI of ulcerated group **(*Dawud et al., 2014*)**.

Table 1: Ulcer scores and descriptive features (*Dawud et al., 2014*).

Score	Features
0	No ulcer
1	Pin point ulcer and changes limited to superficial layers of mucosa
2	Ulcer less than 1mm in size
3	Ulcer more than 1 mm but less than 2 mm in size
4	Ulcer more than 2 mm or perforated ulcer

After these analyses, specimens were taken from fundic area. Then, they were immediately fixed in 10% formol saline and processed for paraffin blocks. Sections of 7 µm thickness were cut and subjected to the followings:

1. Haematoxylin and eosin (H & E) stain ***(Kiernan, 2001)***.
2. Periodic acid-schiff (PAS) reaction for demonstration of polysaccharides ***(Kiernan, 2001)***.
3. Immunohistochemical detection of Caspase -3 as a marker of apoptosis ***(Said et al., 2004)***.
4. Immunohistochemical detection of PCNA as a marker of cell proliferation ***(Tiwari et al., 2016)***.

Determination of stomach Malondialdehyde (MDA):

MDA level was assessed in tissue homogenate at the Biochemistry department of Kasr ElAiny University according to ***Ohkawa et al., (1979)***.

I -Histological staining

Hematoxylin & Eosin staining *(Kiernan, 2001)*.

II-Immunohistochemical staining *(Suvarna et al., 2012)*

Morphometric Study:

Using the image analyzer computer system (Leica Qwin 500 LTD, Cambridge, UK), the following parameters were measured:

a. The mean area % of PAS reaction.

b. The mean area % of PCNA and caspase 3 immunostaining.

Image analyzer study:

Data were obtained using "Leica Qwin 500 C" image analyzer computer system Ltd. (Cambridge, England). The image analyzer consisted of a coloured video camera, coloured monitor, hard disc of IBM personal computer connected to the microscope, and controlled by "Leica Qwin 500 C" software.

The image analyzer was first calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units. Slides were examined under the light microscope.

Statistical Analysis:

Quantitative data were expressed as group mean ± SD. The statistical analysis was carried out using ANOVA, with SPSS version 16 (SPSS, Chicago, IL, USA) ***(Emsley et al., 2010).***

3. Results

a- Ulcer score index (UI) and preventive index:

The ulcer (INDO) group had an ulcer score of 20.5 ± 1.7 and UI of 2050. Treated rats with ESP, CoQ10, and EP prior to INDO administration showed significant decrease in ulcer scores. Rats in the ESP group (low dose) had an ulcer score of 15.2±3.1while that of the ESP group (high dose) had a mean score of 3± 0.03. CoQ10 and EP groups had a value of 10± 0.2 and 9± 0.32 respectively. Based on the calculated preventive index, high dose of ESP appeared to have a greater gastro-protective effect, with a group UI of 300 and a net preventive index of 85.3%. In comparison, rats of CoQ10 group had UI of 1000 and a net preventive index of 51.2%, while that of EP group had UI of 900 with a net preventive index of 56%. The low dose of ESP had the least net preventive index 25.8% (Table 2).

Table 2: Mean±SD of ulcer score, index and the net preventive index.

Groups	*Ulcer score*	*Ulcer index*	*Net preventive index*
Control	----	-----	----
INDO	20.5±1.7	2050*	0
ESP (a)+INDO	15.2±3.1	1520*#	25.8%
ESP (b)+INDO	3±0.03	300*#@	85.3%
CoQ10+INDO	10±0.2	1000*#@	51.2%
EP+INDO	9±0.32	900*#@	56%

* Significant difference compared to control.
# Significant difference compared to INDO.
@ Significant difference compared to ESP (b)+INDO.

b- Determination of oxidative status (measurement of Malondialdehyde MDA) level in tissue homogenate:

Table 3: Mean±SD of MDA level

Groups	**MDA** (mmol/gm)
Control	**13.8±1.2**
INDO	**72.5±3.3***
ESP (a)+INDO	**48.4±5.1*#@**
ESP (b)+INDO	**20**.8±**2.1*#**
CoQ10+INDO	**35.3±4.2*#@**
EP+INDO	**29.5±2.6*#@**

* Significant difference compared to control.
# Significant difference compared to INDO.
@ Significant difference compared to ESP (b)+INDO.

Histological and immunohistochemical results

Group I (control group):

H & E examination of the fundic sections of the stomach of the control rats showed the normal architecture of the fundic mucosa. Surface mucus cells appeared polyhedral with basal oval nuclei. The gland opened by pits which appeared short, narrow, perpendicular to the surface. The glands were formed of isthmus lined with surface mucus cells, neck lined with parietal cells and mucus neck cells, and base lined with apparently normal peptic and parietal cells. Parietal cell appeared polyhedral with rounded pale vesicular nuclei and acidophilic cytoplasm. The bases of fundic glands showed normal appearance of basophilic peptic cells. Muscular is mucosa was also seen **(Figs. 5-7).**

The examined fundic section showed strong PAS-positive mucus in surface columnar mucus-secreting cells and extending down into the fundic pits. PAS-positive mucus was also seen in the neck region **(Fig. 8)**.

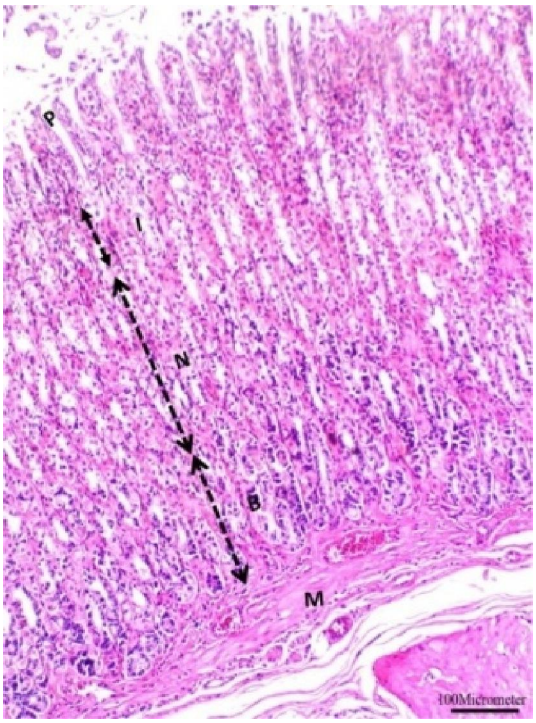

Fig. (5): A photomicrograph of fundic section of stomach of the control rats showing the normal architecture of the fundic mucosa and fundic gland. The gland opens by short narrow pits (P) perpendicular to the surface. Fundic glands are divided into isthmus (I), neck (N) and base (B). Muscularis mucosa (M) is also seen.
(H & E, X100).

The examined fundic section showed faint positive cytoplasmic immunoreaction for caspase-3 in some cells of fundic gland **(Fig. 9)**.

The examined fundic section showed a strong positive nuclear immunoreaction for PCNA mainly in the isthmus region. Moderate reaction was seen in the neck region. Few cells in the basal region showed also moderate nuclear reaction **(Fig. 10)**.

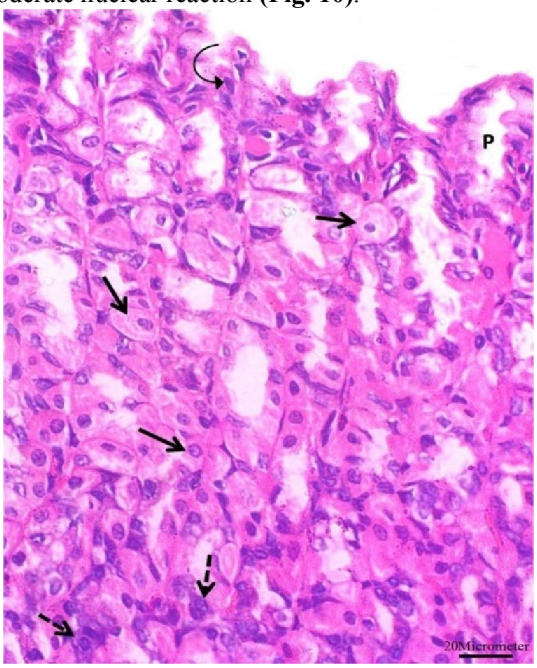

Fig. (6): A photomicrograph of fundic section of a control rat showing fundic glands lined by surface columnar mucus secreting cells (curved arrow) and polyhedral parietal cells with rounded pale vesicular nuclei and acidophilic cytoplasm (thin arrows). Note the short gastric pits (P) and some peptic cells (interrupted arrows).

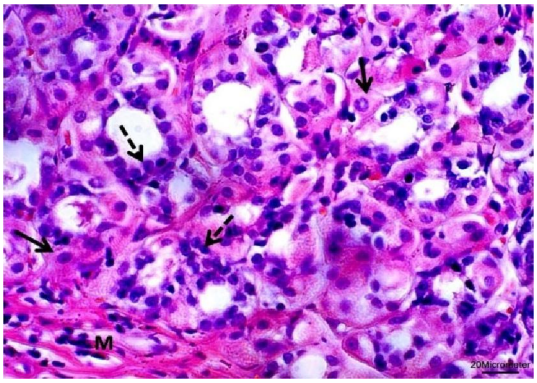

Fig. (7): A photomicrograph of fundic section of a control rat showing basal parts of fundic glands with normal appearance of basophilic peptic cells (interrupted arrows) and polyhedral parietal cells (thin arrows). **(H & E, X400).**

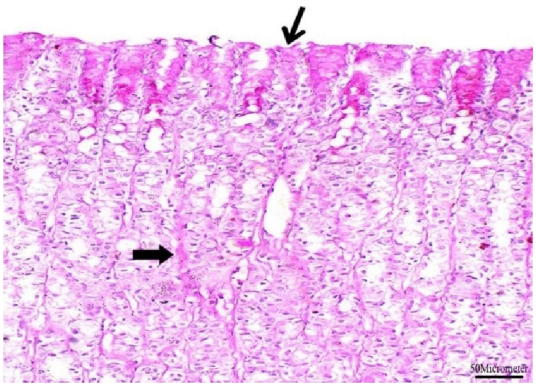

Fig. (8): A photomicrograph of fundic section of the control group showing strong PAS-positive mucus in surface columnar mucus-secreting cells and extending down into the fundic pits (arrow). PAS-positive mucus is also seen in the neck region (thick arrow). **(PAS, X200).**

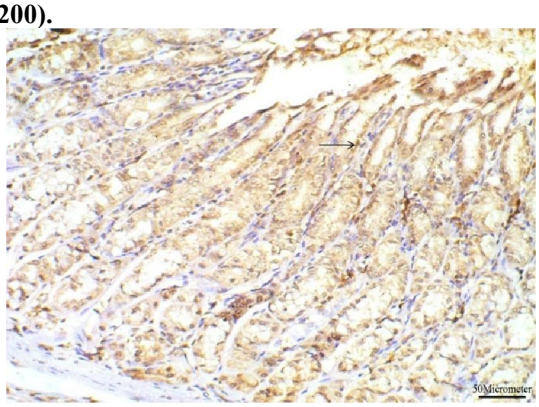

Fig. (9): A photomicrograph of fundic section of the control group showing faint positive cytoplasmic immunoreaction for caspase-3 in some cells of fundic gland (arrow). **(Caspase, X200).**

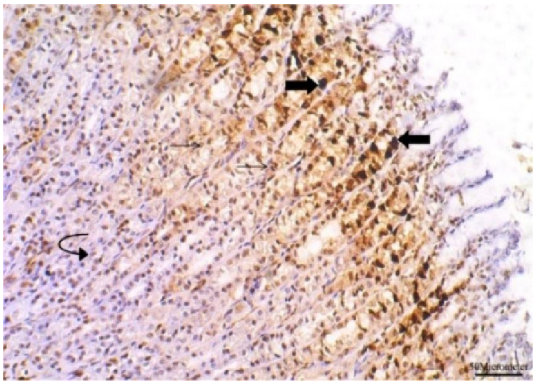

Fig. (10): A photomicrograph of fundic section of the control group showing a strong positive nuclear immunoreaction for PCNA mainly in the isthmus region (thick arrows). Moderate reaction is seen in the neck region (thin arrows). Few cells in the basal region show also moderate nuclear reaction (curved arrow). **(PCNA, X200).**

Group II (Indomethacin-only group (INDO)):

Examination of the fundic sections showed disorganization of normal architecture of fundic gland, desquamation, sloughing and multiple deep erosions reaching down to muscular is mucosa. The surface epithelium was denuded. The surface columnar mucus-secreting cells were lost. There was disorganization of the normal architecture of the fundic glands. Widening of lumen of some glands was noticed. Many parietal cells appeared ballooned, vacuolated with eccentric pyknotic nuclei. Congestion of blood vessels in the mucosa with extravasation of RBCs was observed. Distorted bases of fundic glands were revealed with degenerative changes of peptic cells and parietal cells. The lamina propria showed mononuclear inflammatory cells **(Figs. 11- 14)**.

The examined fundic section showed absence of PAS-positive mucus in the damaged areas of mucosa with its presence in the neck region**) Fig. 15)**.

The examined fundic section showed strong and widespread positive cyctoplasmic immunoreaction for caspase-3**(Fig. 16)**.

The examined fundic section showed moderate positive PCNA immunoreactions **(Fig. 17)**.

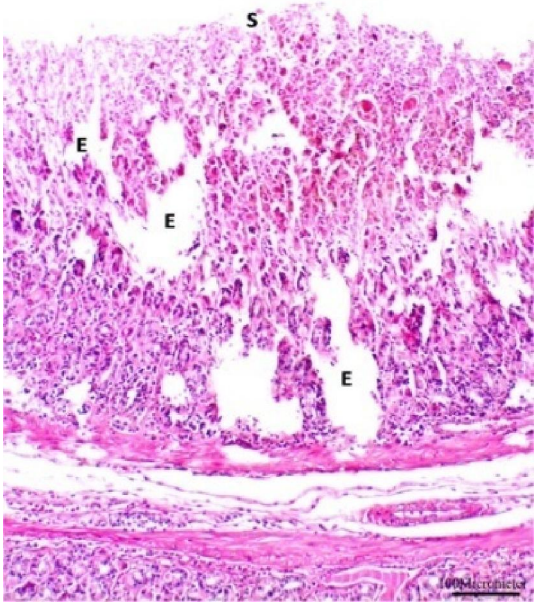

Fig. (11): A photomicrograph of fundic section in INDO group showing disorganization of the normal architecture of fundic mucosa, desquamation and sloughing (S) of surface epithelium. Multiple deep erosions (E) reaching down to muscularis mucosa are noted. **(H & E, X100).**

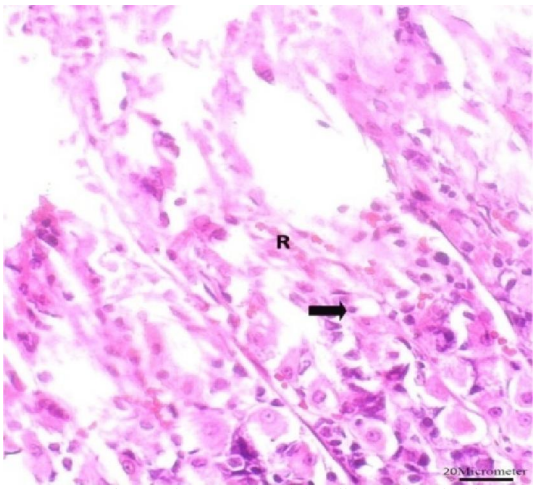

Fig. (12): A photomicrograph of fundic section in the INDO group showing the denuded surface epithelium. The surface columnar mucus-secreting cells are lost. Many parietal cells appear with vacuolated cytoplasm and eccentric pyknotic nuclei (thick arrow). Extravasated RBCs are seen (R). **(H & E, X400)**

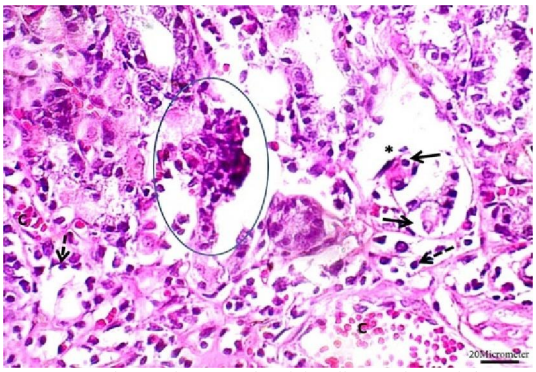

Fig. (13): A photomicrograph of fundic section of the INDO group showing distorted bases of fundic glands with degenerative changes of peptic cells (interrupted arrows) and parietal cells (thin arrows). Widening of lumen of some glands (*). The lamina propria shows inflammatory cells (circle). Congestion of blood vessels in the mucosa is noticed (C). **(H & E, X400).**

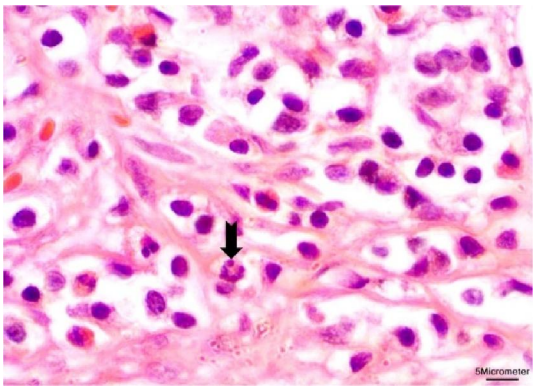

Fig. (14): Higher magnification of the previous section showing mononuclear inflammatory cells (pointed arrow). **(H & E, X1000).**

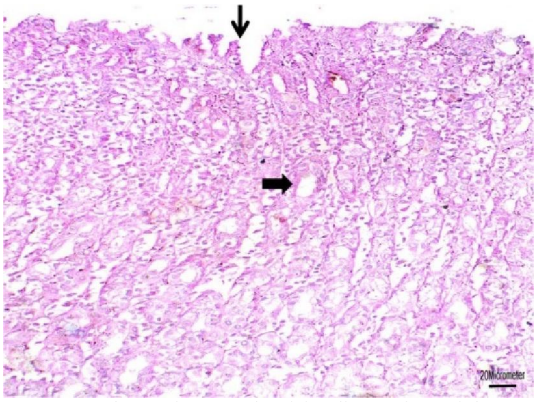

Fig. (15): A photomicrograph of fundic section of the INDO group showing absence of PAS-positive mucus in the damaged areas of mucosa (arrow) with its presence in the neck region (thick arrow). **(PAS, X200).**

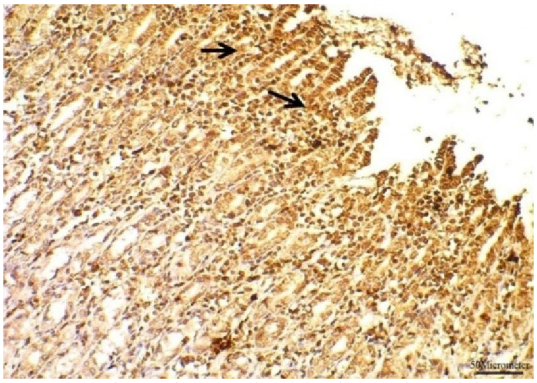

Fig. (16): A photomicrograph of fundic section of the INDO group showing strong and widespread positive cyctoplasmic immunoreaction for caspase-3 (arrows).
(Caspase, X200).

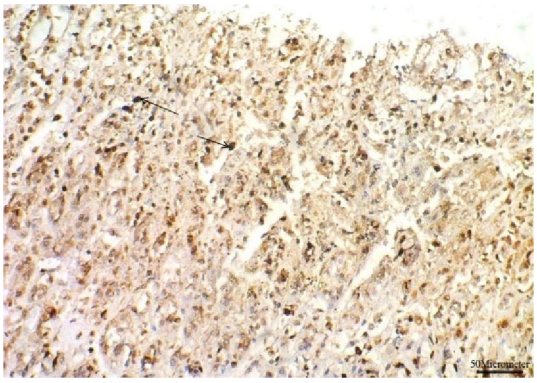

Fig. (17): A photomicrograph of fundic section of the INDO group showing moderate positive PCNA immunoreactions (arrows). **(PCNA, X200).**

Group III (CoQ10 group+INDO):

Examination of the fundic sections showed restoration of the normal architecture of the fundic mucosa. The thickness of the mucosa was apparently similar to that of the control group. Mucosa showed some areas with normal surface columner mucus secreting cells. Other areas showed flattened surface epithelium. The fundic gland opened by short narrow pits that were perpendicular to the surface. The glands were divided into isthmus, neck and base. There was widening of some glands. Some parietal cells were with normal appearance, others with vacuolated cytoplasm and eccentric darkly stained nuclei. There were extravasated RBCs in the lamina propria. Few areas of widening in the bases of the glands were noticed. Restored organization of bases of fundic glands was observed with some normal peptic cells and others with vacuolated cytoplasm. Muscularis mucosa was also seen **(Figs. 18- 20)**.

The examined fundicsection showed PAS-positive reaction in surface columnar mucus-secreting cells and extending down into the fundic pits. The neck region was also containing PAS-positive mucus **(Fig. 21)**.

The examined fundicsection showed a strong cytoplasmic widespread positive immunoreaction for caspase-3**(Fig. 22)**.

The examined fundicsection showed strong positive nuclear immunoreaction in the isthmus region and neck regions of the glands. Few cells in basal parts showed moderate positive reaction **(Fig. 23)**.

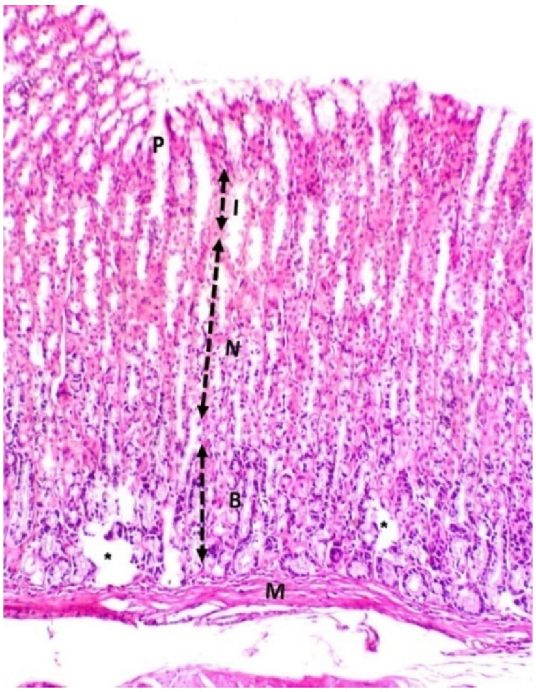

Fig. (18): A photomicrograph of fundic section of the CoQ10 group showing restoration of the normal architecture of the fundic mucosa with short narrow pits (P) which are perpendicular to the surface. Fundic glands are divided into isthmus (I), neck (N) and base (B). Muscularis mucosa (M) is also seen. The thickness of the mucosa is apparently similar to that of the control group. Few areas of widening in the bases of the glands are noticed (*). **(H & E, X100).**

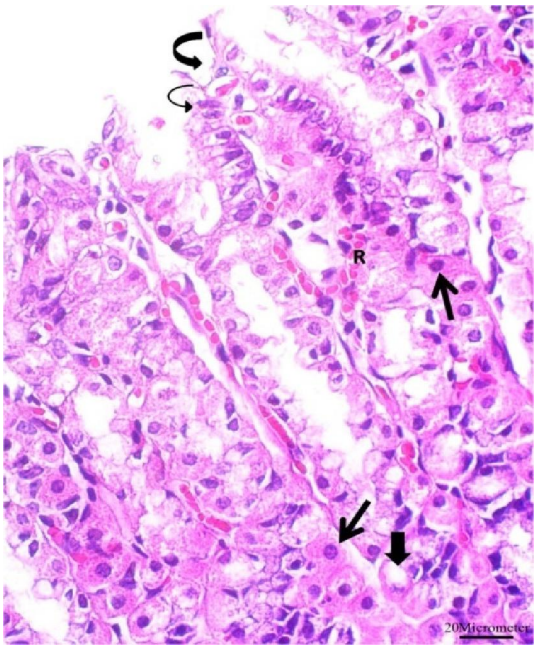

Fig. (19): A photomicrograph of fundic section of the CoQ10 group showing some areas with normal surface columner mucus secreting cells (curved arrow). Other areas show flattened surface epithelium (thick curved arrow). Some parietal cells appear polyhedral with acidophilic cytoplasm and vesicular nuclei (thin arrows), others have vacuolated cytoplasm and eccentric darkly stained nuclei (thick arrow). Note extravasated RBCs in the lamina propria (R). **(H & E, X400).**

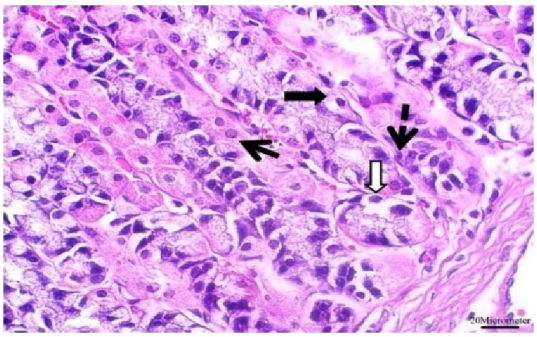

Fig. (20): A photomicrograph of lower part of fundic gland of the CoQ10 group showing some parietal cells with normal appearance (thin arrow). Others have vacuolated cytoplasm and darkly stained nuclei (thick black arrow). Restored organization of bases of fundic glands is observed with some normal peptic cells (interrupted arrow) and others with vacuolated cytoplasm (thick white arrow). **(H & E, X400).**

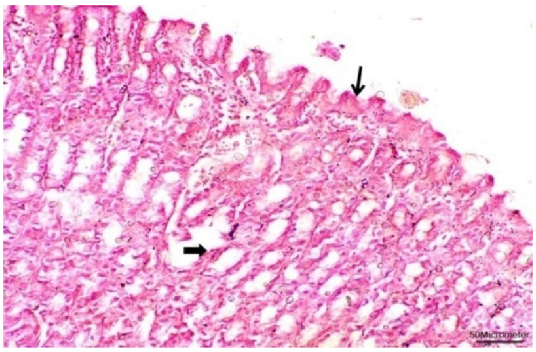

Fig. (21): A photomicrograph of fundic section of the CoQ10 group showing PAS-positive reaction in surface columnar mucus-secreting cells and extending down into the fundic pits (arrow). The neck region is also containing PAS-positive mucus (thick arrow). **(PAS, X200).**

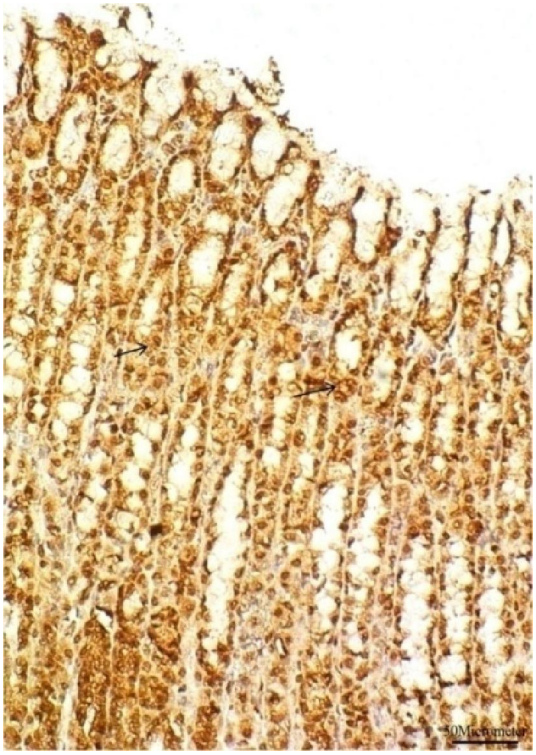

Fig. (22): A photomicrograph of fundic section of the CoQ10 group showing a strong cytoplasmic widespread positive immunoreaction for caspase-3 (arrows).
(Caspase, X200).

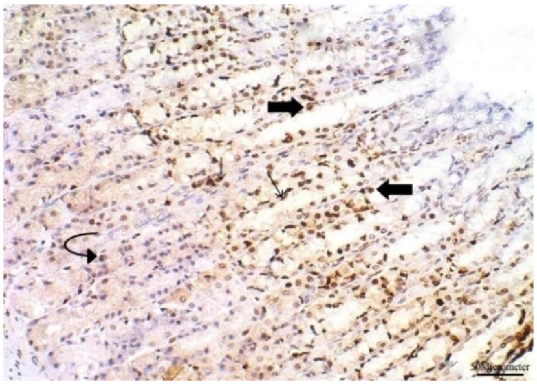

Fig. (23): A photomicrograph of fundic section of the CoQ10 group showing strong positive nuclear immunoreaction in the isthmus (thick arrows) and neck regions of the glands (thin arrows). Few cells in basal parts show moderate positive reaction (curved arrow).
(PCNA, X200).

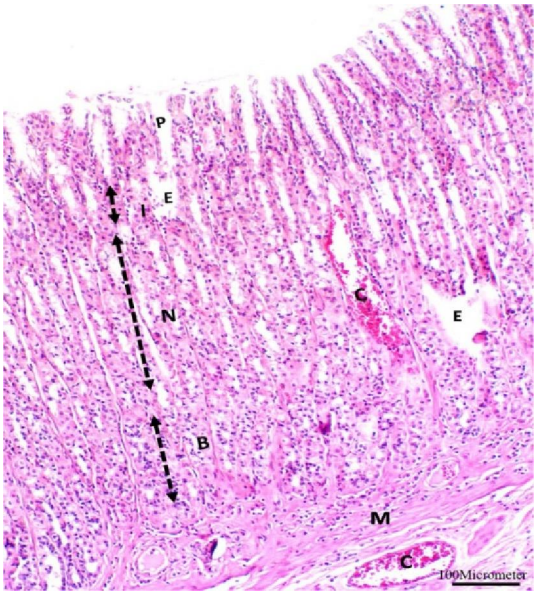

Fig. (24): A photomicrograph of fundic section of the primrose group showing restoration of the normal architecture of gastric mucosa. Fundic glands are divided into isthmus (I), neck (N) and base (B). Muscularis mucosa (M) is also seen. The thickness of the mucosa is apparently similar to that of the control group. The fundic pits (P) show slight widening. Areas of erosion are noticed (E). Congested blood vessels are seen (C). **(H & E, X100).**

Group IV (Evening primerose+INDO group (EP)):

Examination of the fundic sections showed restoration of the normal architecture of gastric mucosa. The thickness of the mucosa was apparently similar to that of the control group. Mucosa showed normal surface columnar mucus-secreting cells. The fundic pits showed slight widening. Fundic glands were divided into isthmus, neck and base. Areas of erosion were noticed. Congested blood vessels were seen. Some parietal cells showed vacuolated cytoplasm with small dark nuclei, others showed vacuolated cytoplasm. Bases of the glands were restored with normal appearance of many parietal cells. Normal appearance of peptic cells was seen. Muscularis mucosa was also seen **(Figs.24- 26)**.

The examined fundicsection showed moderate PAS-positive reaction in surface columnar mucus-secreting cells extending down into the fundic pits and in the neck region **(Fig. 27)**.

The examined fundicsection showed a strong widespread positive cytoplasmic immunoreaction for caspase-3 **(Fig. 28)**.

The examined fundicsection showed strong nuclear positive immunoreaction in the isthmus region and extending to the neck region of the gland. Few cells in the basal parts showed moderate positive reaction **(Fig.29)**.

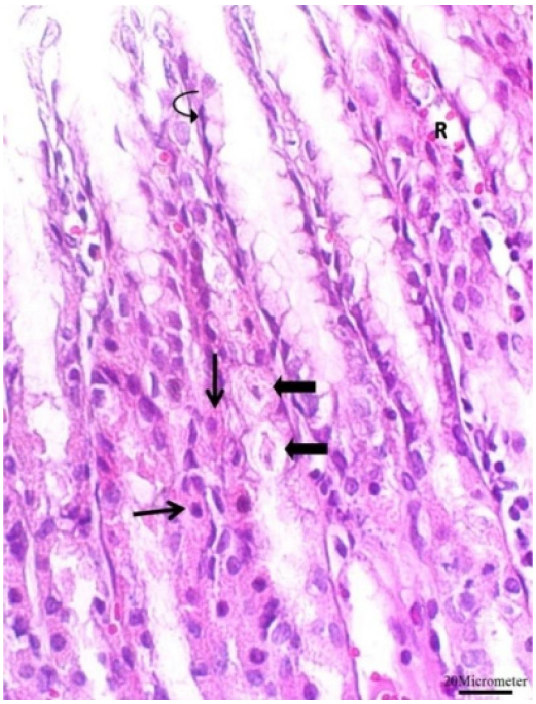

Fig. (25): A photomicrograph of upper part of fundic section of the EP group showing normal surface columnar mucus-secreting cells (curved arrow). Some parietal cells show normal appearance (thin arrows). Few cells show vacuolated cytoplasm with small dark nuclei (thick arrow). Extravasated RBCs are noticed (R). **(H & E, X400)**

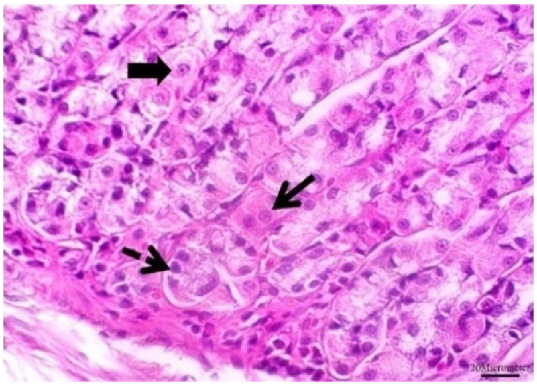

Fig. (26): A photomicrograph of lower part of fundic gland of the EP group showing restored bases of the glands with normal appearance of many parietal cells (thin arrow). Some parietal cells show vacuolated cytoplasm (thick arrow). Note normal appearance of peptic cells (interrupted arrow). **(H & E, X400).**

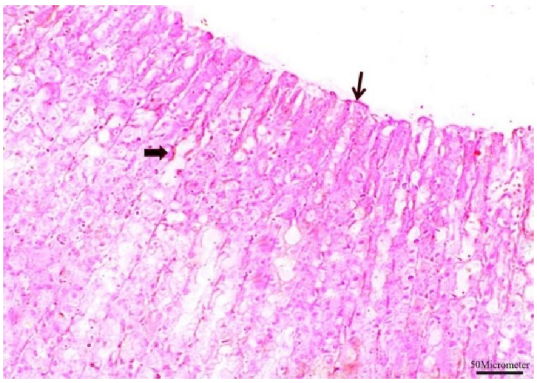

Fig. (27): A photomicrograph of fundic section of the EP group showing moderate PAS-positive reaction in surface columnar mucus-secreting cells extending down into the fundic pits (arrow) and in neck region (thick arrow). **(PAS, X200).**

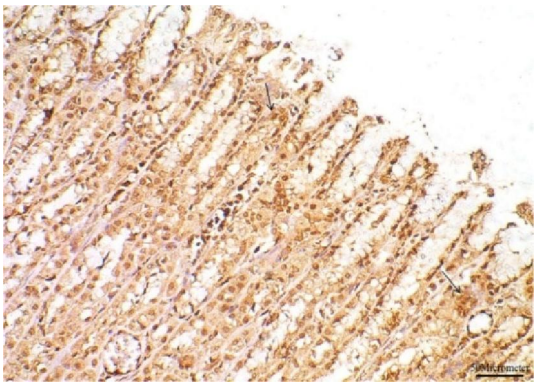

Fig. (28): A photomicrograph of fundic section of the EP group showing a strong widespread positive cytoplasmic immunoreaction for caspase-3 (arrows). **(Caspase, X200).**

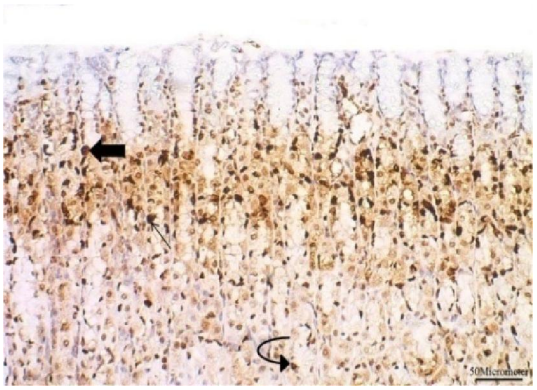

Fig. (29): A photomicrograph of fundic section of the EP group showing strong nuclear positive immunoreaction in the isthmus region (thick arrow) and extending to the neck region of the gland (thin arrow). Few cells in the basal parts show moderate positive reaction (curved arrow). **(PCNA, X200).**

Group Va) ESP low dose +INDO (ESP a) (:

Examination of the fundic sections showed restoration of the normal architecture of mucosa. Mucosa showed normal appearance of surface columnar mucus-secreting cells. Mucosa showed short narrow pits that were perpendicular to the surface. Fundic glands were divided into isthmus, neck and base. Marked widening of the fundic glands and many areas with erosions were seen. Mononuclear inflammatory cell infiltrate was seen in lamina propria. Distorted bases of the glands were noticed. Most parietal cells and peptic cells showed vacuolated cytoplasm and small darkly stained nuclei. Muscularis mucosa was also seen **(Figs. 30- 33)**.

The examined fundicsection showed mild PAS positive reaction in surface columnar mucus-secreting cells extending down into the fundic pits and in neck region **(Fig. 34)**.

The examined fundicsection showed a strong widespread positive cytoplasmic reaction for caspase-3 **(Fig. 35)**.

The examined fundicsection showed moderate nuclear immunoreaction for proliferating cell nuclear antigen (PCNA) located at the isthmus region. Few cells in the basal parts showed mild reaction **(Fig. 36)**.

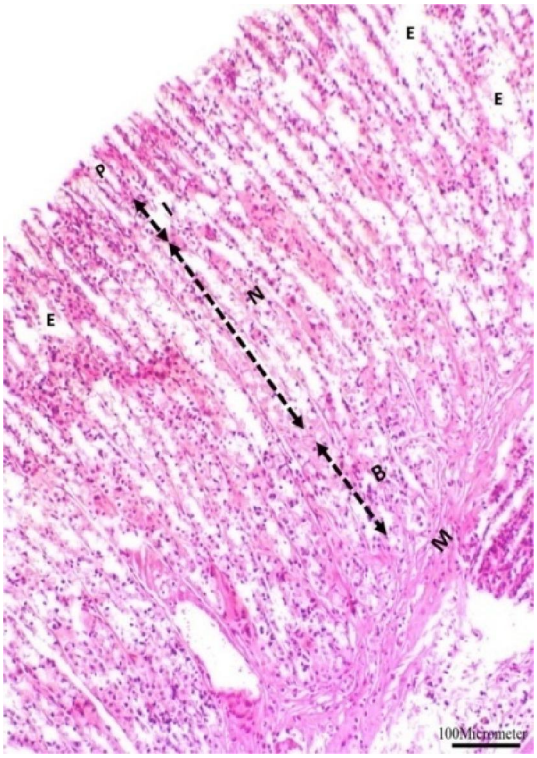

Fig. (30): A photomicrograph of fundic section of the ESP (a) group showing restoration of the normal architecture of mucosa with short narrow pits (P) that are perpendicular to the surface. Fundic glands are divided into isthmus (I), neck (N) and base (B). Muscularis mucosa (M) is also seen. Marked widening of the fundic glands and many areas with erosions are seen (E). **(H & E, X100).**

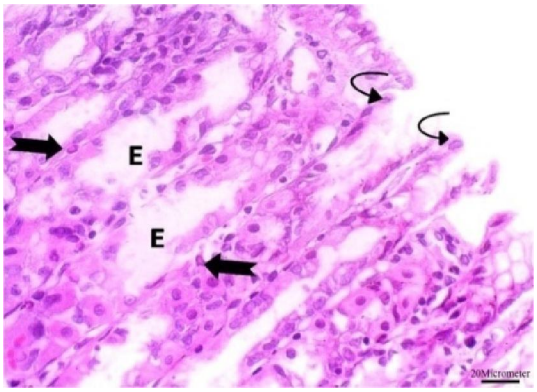

Fig. (31): A photomicrograph of upper part of fundic section of the ESP (a) group showing normal appearance of surface columnar mucus-secreting cells (curved arrows). Areas of erosions are noted (E). Mononuclear inflammatory cell infiltrate are seen in lamina propria (pointed arrows). **(H & E, X400).**

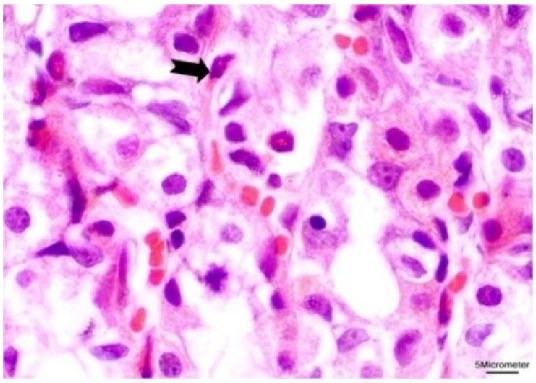

Fig. (32): A photomicrograph of upper part of fundic section of the ESP (a) group showing mononuclear inflammatory cell infiltrate (pointed arrow). **(H & E, X1000).**

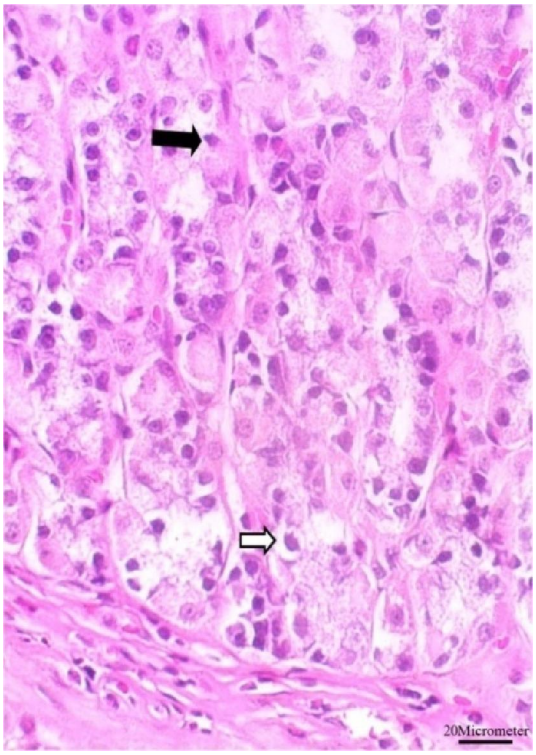

Fig. (33): A photomicrograph of lower part of fundic gland of the ESP (a) group showing distorted bases of the gland. Most parietal cells show vacuolated cytoplasm (thick black arrow). Peptic cells have vacuolated cytoplasm and small darkly stained nuclei (thick white arrow). **(H & E, X400).**

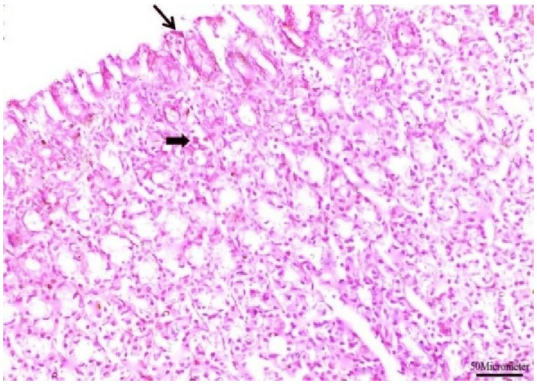

Fig. (34): A photomicrograph of fundic section of the ESP (a) group showing mild PAS positive reaction in surface columnar mucus-secreting cells extending down into the fundic pits (arrow) and in neck region (thick arrow). **(PAS, X200).**

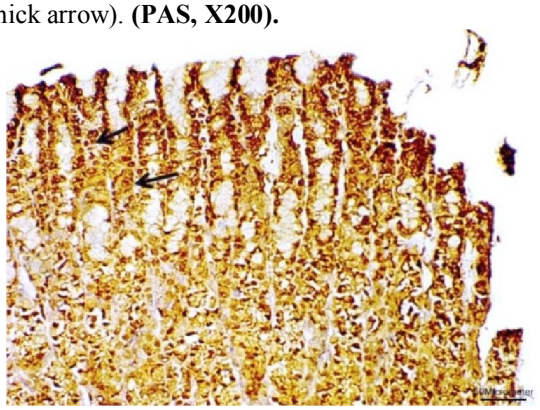

Fig. (35): A photomicrograph of fundic section of the ESP (a) group showing a strong widespread positive cytoplasmic reaction for caspase-3 (arrows). **(Caspase, X200).**

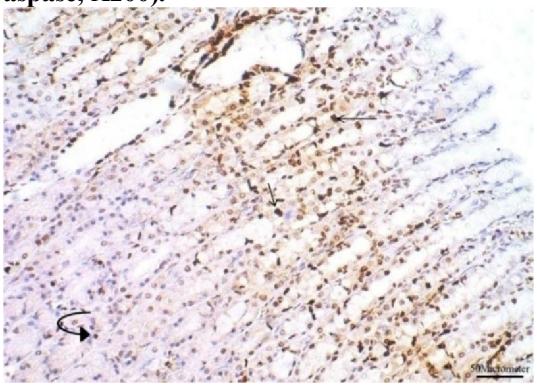

Fig. (36): A photomicrograph of fundic section of the ESP (a) group showing moderate nuclear immunoreaction for PCNA located at the isthmus region (arrows). Few cells in the basal parts show mild reaction (curved arrow). **(PCNA, X200).**

Group Vb (ESP high dose+INDO (ESPb)):

Examination of the fundic sections showed normal architecture of fundic mucosa. The thickness of the mucosa was apparently similar to that of the control group. Surface columnar mucus-secreting cells were normal. The fundic pits showed slight widening. Fundic glands were divided into isthmus, neck and base. Some parietal cells showed vacuolated cytoplasm with small dark nuclei were observed. Bases of most of the glands were restored with normal appearance of peptic cells and parietal cells. Muscularis mucosa was also seen **(Fig. 37- 39)**.

The examined fundicsection showed strong PAS-positive reaction in surface columnar mucus-secreting cells extending down into the fundic pits. The neck region showed also moderate PAS-positive mucus **(Fig. 40)**.

The examined fundicsection showed a moderate positive cytoplasmic immunoreaction for caspase-3 in the upper part of gland and mild immunoreactions in lower part **(Fig. 41)**.

The examined fundicsection showed a strong positive nuclear immunoreaction in the isthmus region, extending to the neck region of the gland **(Fig. 42)**.

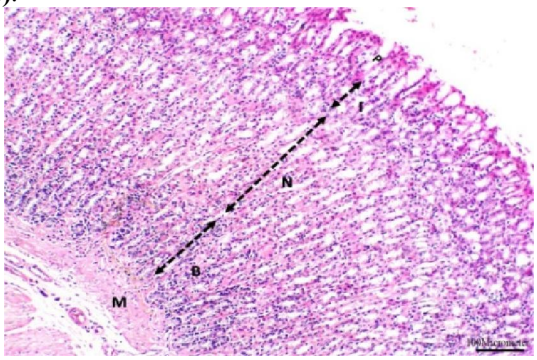

Fig. (37): A photomicrograph of fundic section of the ESP (b) group showing normal architecture of fundic mucosa. Fundic glands are perpendicular to the surface and are divided into isthmus (I), neck (N) and base (B). Muscularis mucosa (M) is also seen. The thickness of the mucosa is apparently similar to that of the control group. The fundic pits (P) show slight widening. **(H & E, X100).**

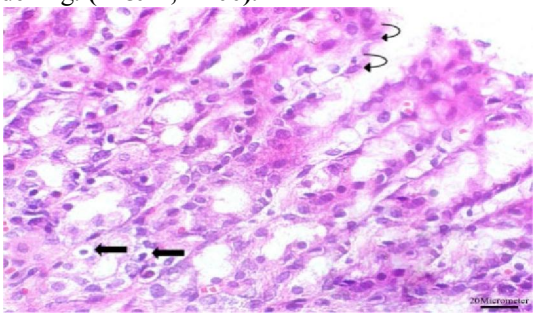

Fig. (38): A photomicrograph of fundic section of the ESP (b) group showing normal surface columnar mucus-secreting cells (curved arrows). Many parietal cells show vacuolated cytoplasm and small dark nuclei (thick arrows). **(H & E, X400).**

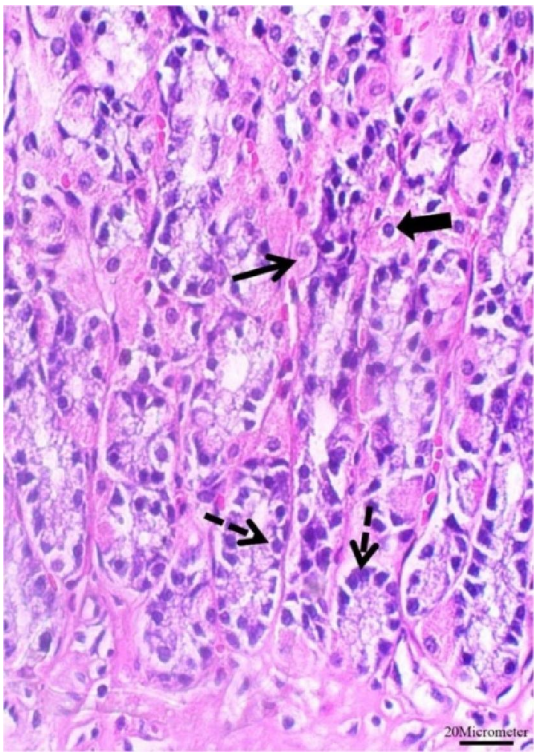

Fig. (39): A photomicrograph of lower part of fundic gland of the ESP (b) group showing restored bases of most of the gland with normal appearance of peptic cells (interrupted arrows) and parietal cells (thin arrow). Some parietal cells with small dark nuclei (thick arrow) are observed. **(H & E, X400).**

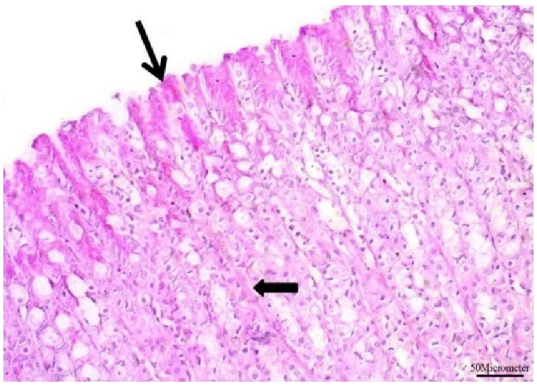

Fig. (40): A photomicrograph of fundic section of the ESP (b) group showing strong PAS-positive reaction in surface columnar mucus-secreting cells extending down into the fundic pits (arrow). The neck region show also moderate PAS-positive mucus (thick arrow). **(PAS, X200).**

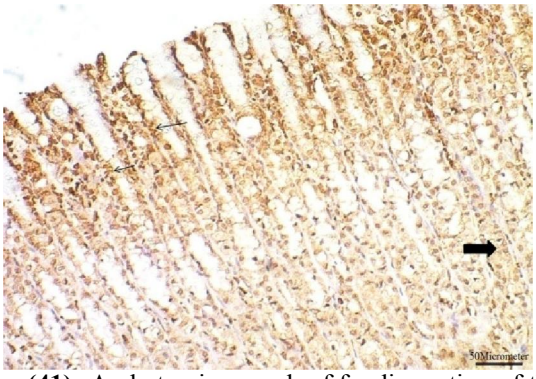

Fig. (41): A photomicrograph of fundic section of the ESP (b) group showing a moderate positive cytoplasmic immunoreaction for caspase-3 in upper part of gland (arrows) and mild immunoreactions in the lower part (thick arrow).
(Caspase, X200).

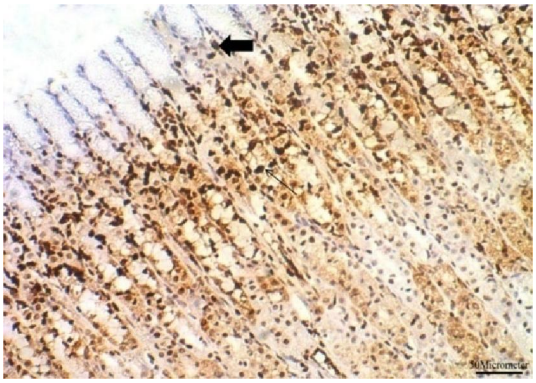

Fig. (42): A photomicrograph of fundic section of the ESP (b) group showing a strong positive nuclear immunoreaction in the isthmus region (thick arrow), extending to the neck region of the gland (arrow). **(PCNA, X200).**

3-Morphometric results

A- Mean area % of PAS +ve reaction (Table 4 & Histogram 1):

1- There was a significant decrease in area % of PAS +ve reaction in INDO group and other groups (CoQ10+INDO, EP+INDO, ESP (a)+INDO and ESP (b)+INDO groups) as compared with the control group ($P < 0.05$). A significant increase in area % of PAS reaction in all groups when compared with INDO group was also revealed ($P < 0.05$).

2- When comparing groups together, the highest value was detected in group ESP (b)+INDO with significant increase in area % of PAS reaction in ESP (b)+INDO rats as compared with ESP (a)+INDO, CoQ10+INDO and EP+INDO groups ($P < 0.05$). There was a significant decrease in CoQ10+INDO group when compared with EP+INDO group ($P < 0.05$). The least value was detected in ESP (a)+INDO

group, with significant decrease was observed as compared with other treatment groups (P < 0.05).

Table 4: Mean ±SD of area% of PAS +ve reaction in all groups:

Group	Control	INDO	CoQ10+INDO	EP+INDO	ESP (a)+INDO	ESP (b)+INDO
Mean±SD	14.7+0.5	2.8+ 0.19*	7.8+0.44*#$@	9.1+0.24*#@	4.6+0.23*#@	13.1+0.25*#

P value significant at <0.05 using ANOVA test. * Significant difference compared to control.
Significant difference compared to INDO. $ Significant difference compared to EP+INDO.
@ Significant difference compared to ESP (b)+INDO.

B- Mean area % of caspase +ve reaction (Table 5 & Histogram 2):

1- There was a significant increase in area % of caspase +ve reaction in INDO group and other groups (CoQ10+INDO, EP+INDO, ESP (a)+INDO and ESP (b)+INDO groups) as compared with control group (P < 0.05). A significant decrease in area % of caspase immunoreaction in all groups as compared with INDO group was also observed (P < 0.05).

2- When comparing the treatment groups together, the least value was detected in ESP (b)+INDO group, with a significant decrease in area % of caspase reaction as compared with CoQ10+INDO, EP+INDO and ESP (a)+INDO groups (P < 0.05). The highest value was detected in ESP (a)+Intergroup. There was a significant increase in caspase reaction of CoQ10+INDO group as compared with EP+INDOgroup (P < 0.05).

Table 5: Mean ±SD of area% of caspase +ve reaction in all groups:

Group	Control	INDO	CoQ10+INDO	EP+INDO	ESP (a)+INDO	ESP (b)+INDO
Mean±SD	14.3+0.43	46.8+2.2*	23.3+2*#@	16.3+0.5*#@	25.6+0.9*#@	12.5+1.1#

P value significant at <0.05 using ANOVA test. * Significant difference compared to control.
Significant difference compared to INDO. @ Significant difference compared to ESP (b)+INDO.

C- Mean area % of PCNA +ve reaction (Table 6 & Histogram 3):

1- There was a significant decrease in area % of PCNA expression in INDO group and other groups (CoQ10+INDO, EP+INDO, ESP (a)+INDO and ESP (b)+INDO groups) as compared with the control group (P < 0.05). A significant increase in area % of PCNA expression in CoQ10+INDO, EP+INDO and ESP (b)+INDO groups as compared with INDO group was revealed (P < 0.05). It worth mentioning that there was a non significant difference between INDO group and ESP (a) +IND group (P < 0.05).

2- When comparing groups together, the highest value was detected in group ESP (b)+INDO with significant increase in area % of PCNA expression as compared with CoQ10+INDO, EP+INDO and ESP (a)+INDO groups (P< 0.05). The least value was detected in ESP (a)+INDO group. There was a significant increase in EP+INDO rats when compared with CoQ10+INDO and ESP (a)+INDO groups (P < 0.05).

Table 6: Mean ±SD of area% of PCNA +ve reaction in all groups:

Group	Control	INDO	CoQ10+INDO	EP+INDO	ESP (a)+INDO	ESP (b)+INDO
Mean±SD	13.1+0.5	3.7+0.6*	6+0.3*#$@	8.5+0.5*#@	4.5+2.5*#$@	14+0.2#

P value significant at <0.05 using ANOVA test. * Significant difference compared to control.
Significant difference compared to INDO. $ Significant difference compared to EP+INDO.
@ Significant difference compared to ESP (b)+INDO.

4. Discussion

Gastric ulcer is one of the most common disorders of the gastrointestinal tract (GIT). It affects 5% of the population around the world, so its prevention and management are considered very important challenges. The NSAIDs are still widely used all over the world for their analgesic and anti-inflammatory properties. Gastric damage induced by NSAIDs is the most common and dangerous side-effect of these drugs. They account for 25% of gastric ulcer cases ***(Boligon et al., 2014and Abourehab, 2017)***.

Indomethacin (INDO) has been documented to have a higher potential to cause gastric injury than other commonly-used NSAIDs. Though the use of newly introduced NSAIDs is increasing day by day, INDO was chosen in this thesis for induction of gastric ulcer as it is still widely used in the remote areas because of its low cost. In addition, it`s one of the

most common NSAIDs that is used for induction of experimental gastric ulcer ***(Tastekin et al., 2018).***

The current treatment of gastric ulcer with anti-secretory drugs, such as PPI and H2 antagonist, as long term treatment is associated with many side effects ***(Almasaudi et al., 2015).***

Therefore, new treatments have been thought 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 ***(DeVault and Talley, 2009)***.

Results of this study showed that INDO-induced gastric ulcer was associated with significant increase in the ulcer index as compared with the control rats. It has been suggested that INDO induces gastric damage via inhibiting the release of protective factors like cyclooxygenase-1, PGE2, bicarbonate and mucus; increasing the aggressive factors like gastric acid; and increasing oxidant parameters while decreasing antioxidant parameters. These results were in agreement with those of ***Sabiu et al. (2016).***

This may be attributed to either free radicals formation or inhibition of synthesis of PGs. Where, decreased PG level may result in impaired gastroprotection, increased gastric acid secretion, decreased blood flow and mucus secretion which are the main etiology of mucosal ulceration ***(Bjarnason et al. 2018).***

A significant decrease in the ulcer index of all groups as compared to INDO group was observed. This decrease may be due to increased protective factors. They restore the balance between the protective and aggressive factors in the stomach or oppose the inhibitory action of INDO on PGE2 synthesis ***(Partick et al., 2018)***. It is worth mentioning that the main action of ESP was through inhibition of the H^+/K^+ ATPase in the parietal cells of the stomach, thus preventing acid secretion. The previous results were in agreement with that of ***El Abhar et al. (2010); Bi et al. (2017) and Kim et al. (2018)*** who revealed antiulcer effect of CoQ10, Primerose and ESP respectively.

Regarding the antioxidant status, there was a significant increase in MDA level in INDO group when compared with other groups. This was in agreement with ***Sabiu et al. (2016); Jambi and Khattab (2019).*** This could be attributed to the increased formation of oxidative stress measures. It has previously been discovered that a decrease in antioxidant enzymes in the stomach induces gastric ulceration. Free radicals initiate MDA which has a major role in the toxicity mechanism of INDO. The damage in gastric mucosa involves an increased level of MDA. If MDA was not scavenged by antioxidant enzymes, this may lead to more accumulation of MDA that cause more tissue damage. So, the unbalance between MDA and antioxidant enzymes leads to oxidative stress and subsequent disturbed cellular functions ***(Sabiu et al., 2016; Jambi and Khattab, 2019).*** The previous results regarding improvement in the treatment groups were consistent with that of ***El Abhar et al. (2010); Bi et al. (2017) and Kim et al. (2018)*** who revealed antiulcer effects of CoQ10, Primerose and ESP respectively.

In H & E stained sections of INDO group, examination of the fundic sections of the stomach showed multiple deep erosions. The surface columnar mucus-secreting cells were lost. There was distorted architecture of fundic glands with congested blood vessels and extravasation of RBCs.

These degenerative changes may be due to PG deficiency which plays an important role in the pathogenesis of NSAID-induced gastric injury. Inhibition of PG synthesis is associated with disturbance of microcirculation, decrease in mucus secretion, lipid peroxidation, and neutrophil activation, or direct cytotoxic effect which are involved in the pathogenesis of gastrointestinal mucosal disorders. Also, the development of the gastric mucosal lesions induced by INDO may also be mediated through generation of oxygen free radicals ***(Kim et al., 2011).***

Others attributed the damaging effect of NSAIDs to low oxygen tension inside the cells and depletion of ATP generation. This consequently affected sodium potassium pump leading to influx of sodium into the cell and an osmotic gain of water with intracellular influx of calcium from the extracellular fluid and its release from intracellular stores. This would activate proteases, phospholipases and endonucleases leading to cellular damage ***(Bjarnason et al. 2018).***

There was inflammatory cellular infiltration. It may be attributed to the neutrophil-endothelial cell interactions as a cause of gastric injury. Dilated glands might be an indication of hypersecretion, which might be related to inhibition of PG I2, a potent anti-secretory agent ***(Zaki and Mohamed, 2014).*** The work of ***Thong-Ngam et al. (2012)*** who suggested that NSAIDs-induced gastric ulceration is a neutrophil-dependent process. He explained that NSAIDs administration to rats caused 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.

The dilated gastric glands that were detected in the current study could be justified by the work of ***Chen et al., (2002)*** who stated that the degeneration of cells lining these gastric glands giving the appearance of wide lumen.

H and E stained sections of the ulcer group also showed apoptotic changes in parietal cells. These findings were in agreement with those of ***Seleem et al., (2010)*** who demonstrated features of degeneration in the oxyntic cells. Their nuclei were shrunken with irregular nuclear membrane and excessive heterochromatin and apparent cytoplasmic vacuolation. Moreover, ***Wallace, (2001)*** related these vacuolations to the increased secretory canalicular membrane elaboration as a result of increased gastric acid secretion due to loss of the inhibitory effect of prostaglandins on the oxyntic cells.

Furthermore, the basal parts of the fundic glands showed apoptotic chief cells. In accordance with those results, ***Al-Sagaaf et al., (2011)*** demonstrated that some areas of the basal region of gastric glands showed loss of architecture and vacuolation of cells whereas other areas showed normal architecture with predominant chief cells full of secretions. They attributed these changes to the aggressive effect of pepsin and lipase of chief cells on the mucosal lining, especially if associated with decreased mucus secretion.

These results were also in agreement with ***Patrick et al. (2018); Minayan et al. (2018) and Oloyede et al. (2018).***

The PAS stained sections revealed a significant decrease in area % of PAS reaction in INDO group as compared with the other groups. The reduced glycoprotein content of the gastric mucosa in INDO group was in agreement with ***Hajrezaie et al. (2015).***

Loss of PAS reaction from the surface cells with its presence in the neck part of the gastric glands denoted increased activity of neck cells in forming new coating mucus to protect the gastric mucosa against further injury (***Zaki and Mohamed 2014).***

The possible mechanism responsible for the topical irritant properties of NSAIDs is their ability to decrease the hydrophobicity of the mucus gel layer in the stomach which is a primary barrier to acid-induced damage in the stomach, via association with the surface active-phospholipids. They suggested that these changes were due to suppressed PG production and damage of the surface epithelial cells and mucus neck cells which led to decreased mucus production. The decrease in mucus 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 PG synthesis, the gastric environment becomes more vulnerable to exogenous (e.g., smoking) or endogenous factors (*H. pylori*, acid, pepsin, bile salts) and, consequently, more prone to develop peptic ulcer and bleeding complications ***(Cryer and Mahaffey, 2014; Alese et al., 2018).***

In the same context, ***Al Asmaria et al. (2016)*** reported that ulcerogenic agents cause dispersal of the gastro-protective mucus 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.

There was a significant increase in area % of caspase reaction in INDO treated group as compared with all other groups. This was in agreement with ***Mard et al. (2016); Shahin et al. (2018)*** who found that INDO significantly up-regulated the expression of caspase-3 by 3.1 fold as compared to the normal group. Oxidative stress and inflammation is a major inducer of cell death by apoptosis through intrinsic pathway caspase-9 then activation of caspase-3. INDO induced apoptosis via upregulation of caspase-9 gene expression leading to increase caspase-3 activity ***(Soliman et al., 2016).***

The mechanism of NSAID-induced apoptosis is believed to be mediated by inhibiting the activity of NF-κB and ubiquitin proteosomes which lead to a common pathway of activation of the apoptotic protease, caspase-3 ***(Mard et al., 2016).***

Regarding degenerative changes, there was a significant decrease in area % of PCNA expression in INDO group as compared with the control group. These could be related to decrease DNA synthesis in the damaged cells ***(Abdelhady and Abdelrahman, 2011).***

Results with INDO group with PCNA were in agreement with ***Yamamoto et al. (2014)*** who found that only few cells were positive for PCNA in the animals treated with INDO alone. Oxidative stress was claimed to have a role in induction of gastric ulcers. The cause of oxidative stress is the increased levels of reactive oxygen species (ROS). Hence, injury to the gastric tissues occurs by damaging membranes and cellular biomolecules such as proteins, DNA and lipids ***(Badr and Al-Mulhim, 2014).*** So, using drugs with anti-oxidant properties could decrease ROS formation and protect gastric tissues from oxidative damage.

CoQ10 was chosen in this study as it is the only endogenously produced lipid with a redox function in mammals. It is a highly lipophilic antioxidant with poor aqueous solubility and slow absorption leading to low and variable oral bioavailability. It was also reported that CoQ10 is the only antioxidant that is fat soluble. Therefore, it was dissolved in olive oil and injected intraperitoneally ***(Papuci et al., 2003)***.

In the current experiment, sections of the CoQ10+INDO group showed appreciable healing of gastric mucosa. It showed restoration of the normal architecture of the fundic mucosa with erosions in few

areas. Some parietal cells were with normal appearance, others with vacuolated cytoplasm and eccentric pyknotic nuclei. There were extravasated RBCs in the lamina propria. There was widening of some glands. Restored organization of bases of fundic glands was observed with some necrotic areas in between. These results were in agreement with ***(Malash et al., 2012; khaleel et al., 2015).***

The healing effect of CoQ10 could be attributed to its ability to hinder INDO-induced ulcer formation and vascular permeability, elevated PGE2, restored the disturbed redox status and boosted nitric oxide level. It was reported that supplementation with CoQ10 in the diet aids healing of chronic gastric ulcers induced by acetic acid in rats *via* inhibiting hypoxia. More recent study reported the potent effectiveness of CoQ10 against gastropathy induced by NSAIDs using the INDO acute gastric ulcer model ***(El Abhar et al., 2010)***. It was reported that its anti-ulcerogenic effect is due to its antioxidant capacity, besides replenishment of PGE2 and nitric oxide in the gastric mucosa; characters that confessed its potential usefulness against gastric damage ***(Malash et al., 2012).***

Moreover, it has electron donating property that may inhibit the decrease in superoxide dismutase in gastric tissue (replenish endogenous antioxidants), hence justifying the preservation of mucosal glutathione levels and inhibiting lipid peroxidation and thereby stabilizes biological membranes. Since mucus production, rapid gastric cell turnover, as well as complete barrier function repair are highly energy dependent processes, thus it is emphasized that adequate energy, besides an intact mitochondria offered by the higher doses of CoQ10, are needed to combat gastric ulceration. Additionally, CoQ10 reduce ROS production and improve epithelial cells function by upregulation of eNOS and hemeoxygenase-1 (HO-1) ***(Malash et al., 2012; Samini et al., 2013; khaleel et al., 2015).***

`A significant increase in area % of PAS reaction in CoQ10+INDO group when compared with INDO group was revealed. This was in agreement with ***Zhang et al. (2018)***. CoQ10 boosted mucin secretion in the gastric juice. Enhancement of mucin secretion may be attributed to increased glutathione and constitutive enhancement of nitric oxide levels in the gastric mucosa. Moreover, gastroprotective mechanisms of CoQ10 were reported via reduction of acidity, with inhibition in peptic activity. Gastric mucus acts as a protective barrier from the noxious effects of both gastric acidity and pepsin and possesses free radical scavenging activity due to its natural composition, so preventing gastric injury induced by majority of insults. Previous study elucidated that an increment in endothelial nitric oxide synthase (eNOS) enhances nitric oxide levels after CoQ10 administration. Moreover, CoQ10 administration raised gastric mucosal PGE2 level partially in INDO-treated rats ***(Malash et al., 2012)***. However, ***El-Abhar et al., (2010)*** found that CoQ10 failed to increase mucus content significantly.

A significant decrease in area % of caspase immunoreaction in CoQ10+INDO group as compared with INDO group was observed. This was in agreement with ***Tsai et al. (2016).*** Previous studies have indicated that CoQ10 attenuated cellular apoptosis by inhibition of mitochondrial depolarization and prevented apoptosis through suppression of mitochondrial dependent caspase-3 protein. This suggested that CoQ10 might improve functional impairment and survival ***(Chen et al., 2013; Tsai et al., 2016).***

Moreover, CoQ10 is a mediator of lipid peroxidation and an essential cofactor in the electron transport chain and a component of the lipid membranes. These effects might explain the notable reduction of the apoptotic cells expressed by marked decrease of caspase-3 expression within tissues ***(Olama et al., 2018).***

A significant increase in area % of PCNA expression in CoQ10+INDO group as compared with INDO group was revealed. CoQ10 may have a proliferative effect as reported by ***Elsheikh et al. (2012); Mazen and Elnegris (2013)***. CoQ10 also might have anti-proliferative effect on cancerous cells as reported by ***Choi et al., (2013)*** using Ki-67 as a marker for proliferation. So CoQ10 might have a modulatory effect according to the present condition. The increased PCNA reactivity accompanied increases in cellular proliferation, which is a sign of ulcer reepithelialisation ***(Elsaed et al., 2018)***.The decrease in caspase as a marker for apoptosis and increase in PCNA as a marker for proliferation indicates that cells tried to proliferate to compensate the ulcer-induced apoptosis.

In the current experiment, Evening Primerose was used to determine its possible healing effect on INDO-induced gastric ulcer. Sections of the EP+INDO group showed appreciable healing of gastric mucosa. Examination of the fundic sections of the stomach showed restoration of the normal architecture of gastric mucosa. The fundic pits showed slight widening. Areas of erosion were also noticed with congested blood vessels. Glands were restored with some parietal cells showing vacuolated cytoplasm.

The previous results were in agreement with ***Dos Santos et al., (2014); Bi et al., (2017).*** The ameliorative effect of EP might be attributed to decreased acid secretion ***(Bi et al., 2017)***. Evevning primerose was found to decrease the serum pepsinogen I level, an atrophic gastritis marker and

pepsinogen II level, a marker of inflammatory response. This results in increase in the pepsinogen I/II ratio ***(Kim et al., 2014)***. On the contrary, ***Cai et al., (2014)*** found that EP did not considerably attenuate the ulcer lesions induced by ethanol or INDO.

A significant increase in area % of PAS reaction in EP+INDO group as compared with INDO group was also revealed. This result was in agreement with ***Cho et al., (2019)*** who revealed improvement in mucus secretion.

A significant decrease in area % of caspase immunoreaction in EP+INDO group as compared with INDO group was observed. This result was in agreement with ***Arimura et al., (2004)*** who demonstrated that rapid increase in intracellular peroxide levels after addition of EP triggers off induction of apoptosis.

A significant increase in area % of PCNA expression in EP+INDO group as compared with INDO group was revealed. This result was not in accordance with ***Lewandowska et al., (2014)*** who reported that EP had anti-proliferative effect on cancerous cells using Ki-67 as a marker for proliferation. Inflammatory conditions are associated with proliferation as a healing process. So, EP might have a modulatory effect rather than proliferative or anti-proliferative effect ***(Kiraly et al., 2015)***. As the ulcer index increased caspase immunostaining increased, while PCNA immunostaining decreased.

In H & E stained sections of ESP (a)+INDO group, examination of the fundic sections of the stomach showed restored architecture of gastric mucosa with marked widening of the fundic glands and some areas with erosions. Neutrophils were seen in lamina propria. Most parietal cells and chief cells showed vacuolated cytoplasm and small darkly stained nuclei. These were in agreement with ***Al-Shaha and Mohammed, (2017).***

The common used therapeutic dose of esomeprazoledoes not exceed 40-80mg/day. ESP even with high dose upto 800-1300mg/ day has no toxic effect. The low dose used in this study (5mg/kg/day) is equivalent to the common used therapeutic dose in clinical medicine ***(Ali and Dalton, 2009).***

In H & E stained sections of ESP (b)+INDO group, examination of the fundic sections of the stomach showed normal architecture of fundic mucosa. The fundic pits showed slight widening. Many parietal cells showed vacuolated cytoplasm and small dark nuclei. Bases of most of the gland were restored with normal appearance of chief cells and parietal cells.

These results were in agreement with ***Narwal et al. (2016); Kim et al. (2018); Sabiu et al. (2016)*** who reported that ESP restored normal appearance. These results were consistent with previous studies which reported the anti-apoptotic effect of ESP in inflammatory conditions **(*Eltahir and Nazmy, 2018*)**.

The present study revealed that ameliorative effect of ESP was dose dependent. Gastric ulceration was almost entirely prevented with ESP at dose 20 mg/kg. Although, previous study done by ***Kim et al., (2018)***, showed that protective effect of ESP on gastric mucosa was not dose-dependent where no additional benefit was seen at the higher ESP dose (40 mg/kg). They reported that effect of ESP was dose dependent below dose of 5mg/kg (human equivalent therapeutic dose) while dose from 5–40mg/kg, there was no significant dose dependent difference in protecting gastric mucosa.

The gastro-protective effects of ESP in combination with NSAIDs are potentially attributed to multiple mechanisms. Both acid-dependent and acid-independent mechanisms were proposed. Esomeprazole inhibited gastric acid secretion in parallel with a reduction in mucosal damage in rats treated with INDO. The acid-independent actions are related to decrease in tissue oxidation and apoptosis and to enhancement of nuclear factor-kB activation ***(Fornai et al., 2011).***

A significant increase in area % of PAS reaction in ESP (a)+INDO group when compared with INDO group was revealed. Also, a significant increase in area % of PAS reaction in ESP (b)+INDO groups when compared with INDO group was revealed. This result was in agreement with ***Almasaudi et al., (2016)*** who detected that treatment with PPI caused preserved gastric mucosal glycoproteins. The present result was also in agreement with ***Alese et al., (2018)*** who detected that the PAS staining intensity of the surface mucus and mucus neck cells in the mucosa was significantly increased upon treatment with PPI.

Likewise, ***El-Ashmawy et al., (2016)*** reported preserved gastric mucosal glycoprotein after PPI treatment. They explained that this could be attributed to its affect in increasing gastric NO level which leads to increased mucus secretion and reduced gastric acid secretion.

A significant decrease in area % of caspase immunoreaction in ESP (a)+INDO group as compared with INDO group was observed. Also, a significant decrease in area % of caspase immunoreaction in ESP (b)+INDO group as compared with INDO group was observed. This was in agreement with ***Mard et al., (2016); Kim et al., (2018)*** who revealed that pretreatment with PPI caused significant reduction in caspase-3.

A significant increase in area % of PCNA expression in ESP (a)+INDO group as compared with INDO group was revealed. Also, a significant increase in area % of PCNA expression in ESP (b)+INDO group as compared with INDO group was revealed.

This was in agreement with ***Kim et al., (2018)***. He suggested that the greatest increase in cell proliferation in response to the increased gastrin drive occurs in the gland compartment of gastric mucosa. As the ulcer index increased caspase immunostaining increased, while PCNA immunostaining decreased.

In the present work, CoQ10+INDO, EP+INDO groups showed better ulcer-healing effects when compared with ESP (a)+INDO group. Gastric glands regained their normal architecture with a thick layer of mucus on the surface, surface mucus cells and mucus neck cells were full of mucus and most of oxyntic cells appeared normal with deep acidophilic cytoplasm.

Regarding comparison between groups concerning PAS, Caspase and PCNA reaction, the best effect was achieved in ESP (b)+INDO group, then EP+INDO group, then CoQ10+INDO group. The worst effect was in ESP (a)+INDO group. It is worth mentioning that we used the highest therapeutic dose of ESP to get better effect, but doses of CoQ10 and EP were considered lowdoses (equivalent to the recommended doses in human) as compared with ESP high dose.

References

1. Abdel-Hady E and Abdel-Rahman G. Protective effect of coenzyme Q10 on cadmium-induced testicular damage in male rabbits. Am-Eurasian J Toxicol Sci 2011; 3:153–160.
2. Abdel-Raheem TI and Bamagous GA. Anti-ulcerogenic effect of genistein against indomethacin induced gastric ulcer in rats. Asian J Pharm Clin Res. 2016; 9 (2): 58-63.
3. Abo-Gresha NM, Abel-Aziz EZand Greish SM. Evening primrose oil ameliorates platelet aggregation and improves cardiac recovery in myocardial-infarct hypercholesterolemic rats. Int J Physiol Pathophysiol Pharmacol. 2014; 6(1):23-36.
4. Abourehab MAS. Peptic ulcer. Peptic Ulcer Disease. 2017; 5: 4-5.
5. Al Asmaria A, Al Shahranib H, Al Masric N, Al Faraidid A, Elfakia I and Arshaduddin M. Vanillin abrogates ethanol induced gastric injury in rats via modulation of gastric secretion, oxidative stress and inflammation. Toxicol Rep. 2016; 3: 105–113.
6. Alese MO, Adewole OS, Ijomone OM, Ofusori DA and Alese OO. Mucus secretion and collagen fibers integrity are compromised in aspirin induced gastric lesion; protective role of Musa paradisiacal. Ital J Anat Embryol. 2018; 123)2(: 136-148.
7. Ali RJ and Dalton HR. Gastrointestinal disease. Aronson JK. Side Effects of Drugs Annual. Elsevier. 2009; 30: 573-587.
8. Almasaudi SB, El-Shitany NA, Abbas AT, Abdel-dayem UA, Ali SS, Al Jaouni SK and Harakeh S. Antioxidant, Anti-inflammatory, and Antiulcer Potential of Manuka Honey against Gastric Ulcer in Rats. Oxid Med Cellul Long. 2015;2016: 1- 10.
9. Al-sagaaf S, Ramadan WS and Abunasef SK. Does commercial aloe vera protects the structure of the gastric mucosa against acute nonsteroidal anti-inflammatory drug (diclofenac sodium)-induced damage? Egyptian J Histol. 2011; 34:80–91.
10. Al-Shaha OM and Mohammed SA. Gastro protective effect of oil extract of Nigella sativa Seeds against Aspirin-Induced Gastric Ulcer in Albino Rats. J Entomol Zool Stud. 2017; 5(4): 725-732.
11. Arimuraa T, Kojima-Yuasaa A, Kennedyb DO and Matsui-Yuasaa I. Reactive oxygen species-independent G1 arrest induced by evening primrose extract in Ehrlich ascites tumor cells. Cancer Letters. 2004; 207(1): 19–25.
12. Badr gm and Al-Mulhim JA. The Protective Effect of Aged Garlic Extract on Nonsteroidal Anti-Inflammatory Drug-Induced Gastric Inflammations in Male Albino Rats. 2014; 2014: 1-9.
13. Bi W, Hu L and Man M. Anti-ulcerogenic efficacy and mechanisms of edible and natural ingredients in NSAID- induced animal models. Afr J Tradit Complement Altern Med. 2017; 14 (4): 221-238.
14. Bjarnason I, Bjarnason I, Scarpignato C, Holmgren E, Olszewski M, Rainsford KD and Lanas A. Mechanisms of Damage to the Gastrointestinal Tract From Nonsteroidal Anti-Inflammatory Drugs. J gastro. 2018; 154(3): 500-514.
15. Boligon AA, Freitas RB, Brum TF, Waczuk EP, Klimaczewski CV, Ávila DS, Athayde ML and Bauermann LF. Antiulcerogenic activity of Scutia buxifolia on gastric ulcers induced by ethanol in rats. Acta Pharm Sinica B. 2014;4(5):358–367.
16. Cai J, Kim T, Jang JY, Kim J, Shin K, Lee S, Choi E, Kim S, Park M, Kim JB and Kim Y. In vitro and in vivo anti-Helicobacter pylori activities of FEMY-R7 composed of fucoidan and evening primrose extract. Lab Anim Res 2014; 30(1): 28-34.
17. Chen CC, Liou SW, Chen WC, Hu FR, Wang IJ and Lin SJ. Coenzyme Q10 rescues ethanol-induced corneal fibroblast apoptosis through the inhibition of caspase-2 activation. J Biol Chem. 2013; 288(17): 11689– 11704.
18. Chen JC, Chiu DF and Chen HM. Role of prostacyclin on microcirculation in endotoxin induced gastroprotection in rats. A Microdialysis study. Shock. 2002; 17(4):334-338.
19. Cho D, Skinner D, Mackey C, Lampkin HB, Elder JB, Lim DJ, Zhang S, McCormick J, Tearney GJ, Rowe SM and Woodworth BA. Herbal dry extract BNO 1011 improves clinical and mucociliary parameters in a rabbit model of chronic rhinosinusitis. International Forum of Allergy & Rhinology. 2019; 9(6): 629-637.
20. Choi H, Park HH, Lee KY, Choi NY, Yu HJ, Lee YJ, Park J, Huh YM, Lee SH and Koh SH. Coenzyme Q10 restores amyloid beta-inhibited proliferation of neural stem cells by activating the PI3K pathway.2013; 1;22(15):2112-2120.
21. Cryer B and Mahaffey KW. Gastrointestinal ulcers, role of aspirin, and clinical outcomes: pathobiology, diagnosis, and treatment. J Multidiscip Healthcare. 2014; 7: 137–146.

22. Dawud FA, Mabrouk MA, Mohammed A and Uma IA. Effect of vitamins C & E on aspirin induced gastric mucosal damage and oxidative stress. Curr Res J Biol Sci. 2014;6(1): 36-41.
23. DeVault KR and Talley NJ. Insights into the future of gastric acid suppression. Nature Rev Gastroenterol Hepatol. 2009; 6(9): 524–532.
24. Dos Santos MM, Olaleye MT, Ineu RP, Boligon AA, Athayde ML, Barbosa NBV and Rocha JBT. Antioxidant and antiulcer potential of aqueous leaf extract of kigella Africana against ethanol-induced ulcer in rats. EXCLI J. 2014;13:323-330.
25. El-Abhar HS. Coenzyme Q10: a novel gastroprotective effect via modulation of vascular permeability, prostaglandin E, nitric oxide and redox status in indomethacin-induced gastric ulcer model, Eur J Pharmacol, Dec.2010; 649(1-3): 314-9.
26. El-Ashmawy NE, Khedr EG, El-Bahrawy HA and Selim HM. Nebivolol prevents indomethacin-induced gastric ulcer in rats. J Immunotoxicol. 2016; 13 (4): 580–589.
27. Elsaed WM, Alahmadi AM, Al-Ahmadi BT, Taha JA and Tarabishi RM. Gastroprotective and antioxidant effects of fluvoxamine on stress-induced peptic ulcer in rats. J Taibah Univ Med Sci. 2018; 13(5): 422-431.
28. El-Sheikh AA, Morsy MA, Mahmoud MM, Rifaai RA and Abdelrahman AM. Effect of coenzyme-q10 on Doxorubicin-induced nephrotoxicity in rats. Adv Pharmacol Sci. 2012; doi: 10.1155/2012/981461. Epub.
29. Eltahir HM and Nazmy MH. Esomeprazole ameliorates CCl4 induced liver fibrosis in rats via modulating oxidative stress, inflammatory, fibrogenic and apoptotic markers. Biomed Pharmacoth. 2018; 97: 1356-1365.
30. Emsley P, Lohkamp B and Scott WG. Features and development of Coot. Acta Crystallogr D Biol Crystallogr. 2010; 66 (4): 486-501.
31. Escobedo-Hinojosa WI, Gomez-Chang E, García-Martínez K, Alquicira RG,2 Cardoso-Taketa A and Romero I. Gastroprotective Mechanism and Ulcer Resolution Effect of Cyrtocarpa procera Methanolic Extract on Ethanol-Induced Gastric Injury. Evidence-Based Complementary and Alternative Medicine. 2018; 4: 1-12.
32. Fornai M, Colucci R, Antonioli L, Awwad O, Ugolini C, Tuccori M, Fulceri F, Natale G, Basolo F and Blandizzi C. Effects of esomeprazole on healing of nonsteroidal anti-inflammatory drug (NSAID)-induced gastric ulcers in the presence of a continued nsaid treatment: Characterization of molecular mechanisms. Pharmacol Res. 2011; 63: 59–67.
33. Hajrezaie M, Salehen N, Karimian H, Zahedifard M, Shams K, et al. Biochanin a gastroprotective effects in ethanol-induced gastric mucosal ulceration in rats. PLoS One. 2015; 10(3): e0121529.
34. Ivanov A, Tokareva O, Gorodetskaya E, Kalenikova E and Medvedev O. Cardioprotection with Intravenous Injection of Coenzyme Q10 is limitedby Time of Administration after Onset of Myocardial Infarction in Rats. J Clin Exp Cardiolog. 2014; 5:4.
35. Jambi HA and Khattab HAH. Potential antioxidant, anti-inflammatory and gastroprotective effect of grape seed extract in indomethacin-induced gastric ulcer in rats. Int. J. Pharmacol. 2019; 15: 209-218.
36. Johnson LR. Gastrointestinal Physiology. Mosby. 2013;8th ed: 176.
37. Khaleel EF, Mostafa DG and Abdel-Aleem GA. Gastroprotective effect of flavonoid quercetin and coenzyme Q10 in indomethacin-induced gastric ulcers in normal and diabetic rats. 2015; 14(12): 58-71.
38. Kim J, Ghosh SR, Weil ACP and Zirkin BR. Caspase-3 and Caspase-Activated Deoxyribonuclease Are Associated with Testicular Germ Cell Apoptosis Resulting from Reduced Intratesticular Testosterone. Endocrinology. 2001; 142(9, 1): 3809-3816.
39. Kim JH, Kim BW, Kwon HJ and Nam SW. Curative effect of selenium against indomethacin-induced gastric ulcers in rats. J Microbiol Biotechnol 2011; 21 (4): 400-4.
40. Kim T, Choi E, Kim J, Shin K, Lee S, Choi Y, Jeon JH and Kim Y. Anti-Helicobacter pylori activities of FEMY-R7 composed of fucoidan and evening primrose extract in mice and humans. Lab Anim Res 2014; 30(3): 131-135.
41. Kim TH, Thapa SK, Lee DY, Chung SE, Lim JY, Jeong HM, Song CH, Choi Y, Cho S, Nam K, Kang W, Shin S and Shin BS. Activity of Oral Administration of Aceclofenac and Esomeprazole in Rats. Pharmacokinetics and Anti-Gastric Ulceration. Pharmaceutics. 2018; 10: 152.
42. Kiraly O, Gong G, Olipitz W, Muthupalani S and Engelward BP. Inflammation-Induced Cell Proliferation Potentiates DNA Damage-Induced Mutations in Vivo. PLoS Genetics. 2015; 11(2): e1004901.
43. Lewandowska U, Owczarek K, Szewczyk K, Sosnowska D, Koziołkiewicz M and Hrabec E. Differentiated impact of procyanidins from evening primrose on human breast cancer cells. Cent Eur J Biol. 2014; 9(6): 647-658.
44. Malash AM, Abdallah DM, Agha AM and Kenawy SA. Gastroprotective Efficacy of Coenzyme. Q10 in Indomethacin-Induced Gastropathy: Other Potential Mechanisms. IOSR J Of Pharmacy.2012;5(3):26-30.
45. Mard SA, Pipelzadeh MH, Teimoori A, Neisi N, Mojahedin S, Khani MZS and Ahmad I. Protective activity of crocin against indomethacin-induced gastric lesions in rats. J Nat Med. 2016; 70:62–74.
46. Mazen NF and Elnegris HM. Role of coenzyme Q10 in testicular damage induced by acrylamide in weaned albino rats: a histological and immunohistochemical study. Egypt J Histol. 2013; 36:164-174.
47. Munir R, Semmar N, Farman M and Ahmad NS. An updated review on pharmacological activities and phytochemical constituents of evening primrose (genus Oenothera). Asian Pac J Trop Biomed. 2017; 7(11): 1046-1054.
48. Narwal S, Saini V and Kaushik V. Evaluatiin of anti-ulcer activity of esomeprazole magnesium trihydrate film in wister rats with gastric ulceration induced by indomethacin. ejpmr. 2016; 3(10): 443-447.

49. Ohkawa H, Ohishi N and Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analyt Biochem. 1979; 95: 351-358.
50. Olama NK, Taha M and Rady HY. The potential protective role of coenzyme q10 on the cyclophosphamide- induced lung toxicity in adult male albino rats: a histological and ultrastructural study. Int J Sci Rep. 2018; 4(9):225-234.
51. Oloyede HOB, Olugbode AE and Salawu MO. Gastroprotective Activity of Fruit Ethanolic Extract of Tetrapleura tetraptera on Indomethacin-Induced Ulcer in Rats. Al-Hikmah J of Pure & Applied Sciences 2018; 6: 20-29.
52. Papucci L, Schiavone N, Witort E, et al. Coenzyme Q10 prevents apoptosis by inhibiting mitochondrial depolarization independently of its free radical scavenging property. J Biol Chem. 2003; 278(30): 28220-28228.
53. Patrick AT, Samson FP and Madusolumuo MA. Antiulcerogenic Effects of Aqueous Stem-Bark Extracts of Pterocarpus erinaceus on Indomethacin-Induced Ulcer in Albino Rats. J Biochem Cell Biol. 2018; 1:2.
54. Sabiu S, Garuba T, Sunmonu ET, Ajani A, Sulyman I and Balogun AN. Indomethacin-induced gastric ulceration in rats: Protective roles of Spondias mombin and Ficus exasperate. Toxicol Rep. 2016; 2: 261-267.
55. Said TM, Paasch U, Glander HJ and Agarwal A. Role of Caspase in male infertility. Hum Reprod. Update. 2004; 10: 39-51.
56. Samini S, Shafaroodi H, Shirazi-Beheshtiha SH, Mosavi Z, and Samini M. A Direct Comparison of Anti-ulcer Effects of Coenzyme Q10 and Vitamin C on Indomethacin-induced Gastric Ulcer in Rat: A Controlled Experimental Study, Journal of Cardio – Thoracic Medicine. 2013; 1(2): 34-40.
57. Seleem HS, Ghobashy HA and Zolfakar AS. Effect of Aspirin versus Aspirin and Vitamin C on Gastric Mucosa (Fundus) of Adult Male Albino Rats. Histological and Morphometric Study. Egypt J Histol. 2010; 33(2):313 – 326.
58. Shahin NN, Abdelkader NF and Safar MM. A Novel Role of Irbesartan in Gastroprotection against Indomethacin-Induced Gastric Injury in Rats: Targeting DDAH/ ADMA and EGFR/ERK Signaling Scientific Reports. 2018; 8:4280.
59. Soliman NA, Zineldeen DH, Katary MA and Ali DA. N-acetylcysteine a possible protector against indomethacin-induced peptic ulcer: crosstalk between antioxidant, anti-inflammatory, and antiapoptotic mechanisms. Can J Physiol Pharmacol. 2016; 14: 1-8.
60. Suvarna KS, Layton C and Bancroft JD. Bancroft's Theory and Practice of Histological Techniques, UK: Elsevier Health Sciences. 2012.
61. Tastekin E, Ayvaz S, Usta U, Aydogdu, Cancilar E and Puyan FZ. Indomethacin-induced gastric damage in rats and the protective effect of donkey milk. Arch Med Sci. 2018; 14(3): 671-678.
62. Thong-Ngam D, Choochuai S, Patumraj S, Chayanupatkul M and Klaikeaw N. Curcumin prevents indomethacin-induced gastropathy in rats. World J Gastroenterol. 2012; 18(13): 1479-1484.
63. Tiwari KP, Bhaiyat MI, Chikweto A, Sharma RN, Younger CH and Pawaiya RVS. Expression pattern of proliferating cell nuclear antigen (PCNA) inround cell tumors of dogs from Grenada, West Indies. Indian J. Vet. Pathol. 2016; 40(2): 144-147.
64. Tsai H, Lin C, Huang P, Li S, Chen J, Lin F, Chen J and Lin S. Coenzyme Q10 Attenuates High Glucose-Induced Endothelial Progenitor Cell Dysfunction through AMP-Activated Protein Kinase Pathways. 2016;4: 1-14.
65. Varga Z, Sabzwari SRA and Vargova V. Cardiovascular Risk of Nonsteroidal Anti-Inflammatory Drugs: An Under-Recognized Public Health Issue. Cureus. 2017; 9(4): e1144.
66. Wallace JL. Pathogenesis of NSAID-induced gastro duodenal mucosal injury. Best practice and research. Clin Gastroenterol. 2001; 15(5): 691-703.
67. Yamamoto A, Itoh T, Nasu R and Nishida R. Sodium alginate ameliorates indomethacin-induced gastrointestinal mucosal injury via inhibiting translocation in rats. World J Gastroenterol. 2014; 20(10): 2641-2652.
68. Zaki SM and Mohamed EA. Effect of glucocorticoids on indomethacin-induced gastric ulcer in the adult male albino rat – histological, morphometric and electron microscopy study. Arch Med Sci. 2014; 10, 2: 381–388.
69. Zhang X, Liu H, Hao Y, Xu L, Zhang T, Liu Y, Guo L, Zhu L and Pei Z. Coenzyme Q10 protects against hyperlipidemia-induced cardiac damage in apolipoprotein E-deficient mice. Lipids in Health and Disease. 2018; 17:279.

10/30/2019