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Original article

The impact of oral ciprofloxacin on the structure and functions of rat gastric mucosa

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ABSTRACT

Ciprofloxacin (CPX), is a fluoroquinolone antibiotic used to treat a number of gram-negative and gram-positive bacterial infections. Ciprofloxacin can cause severe side effects, ranging from tendon problems, nerve damage, to serious mood or behavior changes.

The purpose of this study was to investigate how ciprofloxacin affects gastric cell lines in rats with a distinctive emphasis on physiological, histopathological, and bacteriological changes. Male albino rats (n = 21) were distributed into three groups; control, CPX, and CPX-withdrawal groups. The treated rats were given CPX tablets (12.5 mg/kg) dissolved in carboxymethyl cellulose (CMC) 0.5% orally once daily via gavage for sixty consecutive days. Control rats received only the vehicle. The withdrawal group was treated for 60 days and the drug was withdrawn for another sixty days. After completion of the experiment, all rats were sacrificed and gastric tissues were treated for light, immunohistochemical, and scanning electron microscopic examination. Image J software was used to measure immune-labeled gastric epithelial cells. Blood samples were also collected for H. Pylori immunoglobulins IgM, IgA, and IgG. Results showed that treated rats acquired significantly strongly positive tumor necrosis factor (TNFα) and significant reduction of serum level of H. pylori IgM, IgA, and IgG in all the study groups. It could be concluded that prolonged oral CPX administration to albino rats changes the gastric mucosal architecture and bacteriology.

1. Introduction

Ciprofloxacin is a broad-spectrum antibiotic that was once used to treat a wide range of infections. It is the first-selected agent used for the management of testicular infections. However, its antibacterial action is achieved by the accumulation of the level of reactive oxygen species (Eskandari et al., 2001; Zhang et al., 2018). In addition, inhibiting a type II topoisomerase (DNA gyrase) and topoisomerase IV leads to bacterial cell death. CPX has many serious side effects including, insomnia, restlessness, seizures, decreased appetite, diarrhea, crystalluria, itching, and tissue damage when given intramuscularly or subcutaneously (Michalak et al., 2017). Relationships among antibiotic usage, the gut community structure, normal physiology and metabolism, individual and public health are still being under research. Alterations in the structure of bacteria, antibiotic resistance genes (ARGs), and mobile genetic components (MGEs) after antibiotic usage are not well-understood (Xu et al., 2020). Infection with H. pylori may be linked with a multiplicity of gastro-duodenal illnesses. However, H. pylori infection is a common peptic ulcer illness and gastric cancer arises in only a lesser minority of diseased people. This study was planned to relate the pathological outcomes with the serological reaction to certain H. pylori antigens in response to CPX use in albino rats (Schumann et al., 2006). Gastric ulcers are the most mutual gastrointestinal and global disorders. They occur predominantly because of the disproportion between the damaging and aggressive causes of the mucosal barrier. The damaging elements comprise stomach hydrochloric acid (HCl), mucosal hypo-perfusion, free oxygen radicals, ethanol, Helicobacter pylori, and extreme

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consumption of non-steroidal anti-inflammatory drugs (NSAIDs) that stimulate stomach mucosal damage and lead to gastric ulceration. The avoidance or management of gastric ulcers is a medicinal challenge. Gastric ulcer treatment has major difficulties, comprising restricted effectiveness of medications against the gastric disorder and severe side effects (AL-Wajeeh et al., 2016). This research aimed to explore whether CPX treatment of rats disturbs gastric epithelial cell biology with definite orientation to the destructive elements of the gastric epithelium, in precisely, H. Pylori which exist deep within the gastric mucus, and the effects of extended local drug use on rat gastric mucosa. H. Pylori contaminates the mucosal lining of the stomach epithelium in about half of the world's population. Contamination with H. pylori leads to gastritis that will progress to chronic inflammation of the mucosal lining of the stomach. This chronic inflammation will progress from chronic atrophic gastritis to metaplasia, dysplasia, and, in 1%–3% of cases to carcinoma of the stomach. This progress from normal to malignant tissues continues and can be repressed by the suppression of the bacteria with antibiotics. CPX may influence the human gut flora even at subtherapeutic levels (Carman and Woodburn, 2001; Butt and Epplein, 2019). H. Pylori infection of the human stomach causes chronic inflammation and practices the highest threat element that progresses to peptic ulcer disease and gastric cancer. Existing regular suppression treatments practice an acid-suppressing medication and two antibiotics, nowadays commonly complemented with bismuth for an additional regular suppression in asymptomatic H. pylori carriers to destroy stomach cancer (Debraekeleer & Remaut, 2018). The mucus coat cover of the gastric epithelium delivers a defensive neutral atmosphere protecting the gastric wall cells; furthermore, it provides a generous niche for H. pylori. It consists of plenty of high-molecular-weight glycoproteins, named mucins, and some lipids, small molecules, and additional proteins. Through disulfide attachment, the mucins form a viscoelastic coat that depresses transmission proportions and is supposed to inhibit the actual transfer of antibiotics (Gotoh et al., 2002).

1.1. Aim of the work

This study was designed to study gastric mucosal physiological, histopathological, immunohistochemical, and bacteriological changes associated with oral CPX administration in male albino rats.

2. Materials and methods

2.1. Research settings

In this study, 21 adult male albino rats were used and divided into three groups: control, experimental, and withdrawal groups.

Group I (Control group): Consisted of seven animals that received carboxy-methyl cellulose (CMC) 0.5 % for sixty consecutive days.

Group II (CPX treated): Consisted of seven animals were received 12.5 mg/kg of ciprofloxacin, dissolved in carboxymethyl cellulose (CMC).

0.5 % orally once daily via gavage for sixty consecutive days. The dosages of CPX were similar to those used in human therapy (Khaki et al., 2008; Reagan-Shaw et al., 2008).

Group III (CPX withdrawal): Consisted of seven animals were received 12.5 mg/kg of ciprofloxacin, dissolved in carboxymethyl cellulose (CMC).

0.5% orally once daily via gavage for sixty consecutive days. The drug was stopped for another sixty consecutive days then animals were sacrificed.

On the sixtieth day, the animals of groups I and II were sacrificed and the third group animals were sacrificed after 60 days from drug withdraw. The regions of the fundus and the body of the stomach were specially chosen as they are the commonest site of drug-induced injuries. These areas were removed by a couple of scissors in-between esophageal and pyloric ends of the gastric body. The gastric mucosa was open by creating a cut along the greater curvature of the stomach (Wallace, 2001; Lim et al., 2019).

2.2. Histological examination

Rat gastric tissues in all groups were collected, washed with saline, and the gastric wall samples were put in 10% buffered formalin for fixation, handled in a tissue processing machine with paraffin-embedded. Prepared for histological assessments, slices of the gastric sections were set at 5 μm thickness and discolored with hematoxylin and eosin (Hx and E) (Bancroft and Gamble, 2008; Tousson, 2016). Other gastric specimens of all groups were dissected, washed with saline, fixed in osmium tetraoxide, and coated with gold-palladium, and photographs were captured by Jeol-scanning electron microscope in Tanta faculty of medicine at electron microscopy unit (Zaki & Mohamed, 2014).

2.3. Immunohistochemical examination

Five μm sections of the stomach wall were immunohistochemically stained to estimate immune-expression of TNFα in the cytoplasm and gastric sections using Avidin-Biotin Complex (ABC) (El-Masry et al., 2020). Proliferating cell nuclear antigen (PCNA) expression in the nuclei of stomach epithelial cells (Calabrese et al., 2004). Concisely, the paraffin-embedded stomach slices were dewaxed and rehydrated. Then the slices were incubated with a monoclonal antibody against TNFα and PCNA (Dako, Carpinteria California, USA); Diaminobenzidine (DAB) was used to demonstrate the immune reaction. The sections were studied for the brownish coloration of DAB reaction and once visualized it was immediately washed off. Mayer's hematoxylin was used as a counterstain. Negative controls were prepared by excluding the primary antibodies. The cells were measured positive if showing a brownish coloring of DAB reaction related to the negative control (Buchwalow and Böcker, 2010; Hasan et al., 2021). The strength of the immunostaining was recorded according to the recording scheme defined by Mori et al. (2003) as severe (+++), moderate (++), mild (+), or nil (−).

2.4. Morphometric study

Image analysis software (Image J, 1.46a, NIH, USA) was used for Immunohistochemical quantification. Randomly selected 10 non-overlapping fields from every slide were measured at the amplification of 400 for measurable assessment of mean zone percentage (calculated as the zone of positive immunohistochemical reaction 100/total area) and mean color intensity of the immunohistochemical reaction of TNFα and PCNA (measured by deducting the color strength of negative immunohistochemical control from the color strength of positively discolored specimens in successive slices) (Emam et al., 2021).

2.5. Laboratory investigations

Measurement of serum H. pylori immunoglobulin titers was useful in the conclusion of H. pylori infection and severity of gastritis in advance with a histopathological examination (Yamamoto et al., 1995). In all groups, blood samples were collected just before the time of scarification for H. Pylori IgM, IgA, and IgG assay with

enzyme-linked immunosorbent assay (ELISA) in Al-Safwa medical laboratory in Tanta.

2.6. Statistical analysis

Quantitative records were assessed with mathematical set SPSS version 22, IBM, Armonk, NY, United States of America. All records were expressed as mean standards ± standard deviation (SD). The standard for statistical importance was significant at the P value < 0.05 for the collected data and highly significant at P value < 0.001. Analysis was done by to one way analysis (ANOVA) to evaluate significant differences between treatment groups (Dawson and Trapp, 2004, Hajrezaie et al., 2012; Altwaijry et al., 2021).

3. Results:

3.1. Histological results

3.1.1. Hx. and E. sections

Control rat slices of the stomach (Group I) presented regularly arranged fundic glands, stomach pits, surface mucous cells, mucous neck cells, parietal cells, chief cells, and connective tissue with underlying submucosa and muscularis mucosa (Fig. 1). CPX treated rat sections of the stomach wall (Group II) revealed widespread damage to the gastric mucosa in the fundus. Moreover, these rats were noticed to declare necrotic changes in the depth of gastric mucosa that reveals widespread leucocyte infiltration and edema of the sub-mucosal layer (Fig. 2). Other sections in the stomach fundus demonstrated honey combing of gastric mucosal glands, hydropic alterations in some gastric cells though other showing small pyknotic nuclei. Vacuolated mucus cells were aggregated giving rosette appearance with intact parietal cells scattered in-between (Fig. 3). Gastric sections of the CPX withdrawal group (Group III) exhibited stomach mucosa with limited necrotic areas. It also revealed submucosal layer with congested blood vessels. They also revealed dilated gastric mucosal glands with limited areas of necrosis. In addition, it exhibits nesting of parietal cells with vacuolated cytoplasm (Fig. 4).

3.1.1.1. TNFα immunohistochemical staining. TNFα -Immunostained sections of the stomach of control rats (Group I) showed mild positive reaction for TNFα expression in the cytoplasm of gastric mucosa (Group I; Fig. 5A). Severe positive reaction for TNFα expression was in the rats treated with CPX (Group II; Fig. 5B) and moderate positive reaction for TNFα expression in the gastric mucosa of the withdrawal group (Group III; Fig. 5C). Statistical analysis of morphometric results showed highly significant (P < 0.001) rise in the area percentage of TNFα positive immunoreaction in group II as linked with the control group. While, group III depicted highly significant (P < 0.001) decrease of both parameters as compared with group II. On the other hand, group III displayed a non-significant (P > 0.05) difference in immunoreactivity as compared to the control group (Table 1).

3.1.1.2. PCNA immunohistochemical staining. PCNA-Immunostained sections of the stomach of control rats (Group I) showed mild positive reaction for PCNA expression in the nuclei of gastric mucosa (Group I; Fig. 6A). Severe positive reaction for PCNA was detected in the gastric mucosa of the rats treated with CPX (Group II; Fig. 6B) and moderate positive reaction for PCNA expression were noticed in the gastric mucosa of the withdrawal group (Group III; Fig. 6C). Statistical analysis of morphometric results showed highly significant (P < 0.001) increase in the area percentage of PCNA positive immunoreaction in group II as related to control animals' group. Meanwhile, rats in group III demonstrated highly significant (P < 0.001) decrease of both parameters as compared with group II. On the other hand, group III revealed a non-significant (P > 0.05) variance in immunoreactivity as linked to the control group (Table 2).

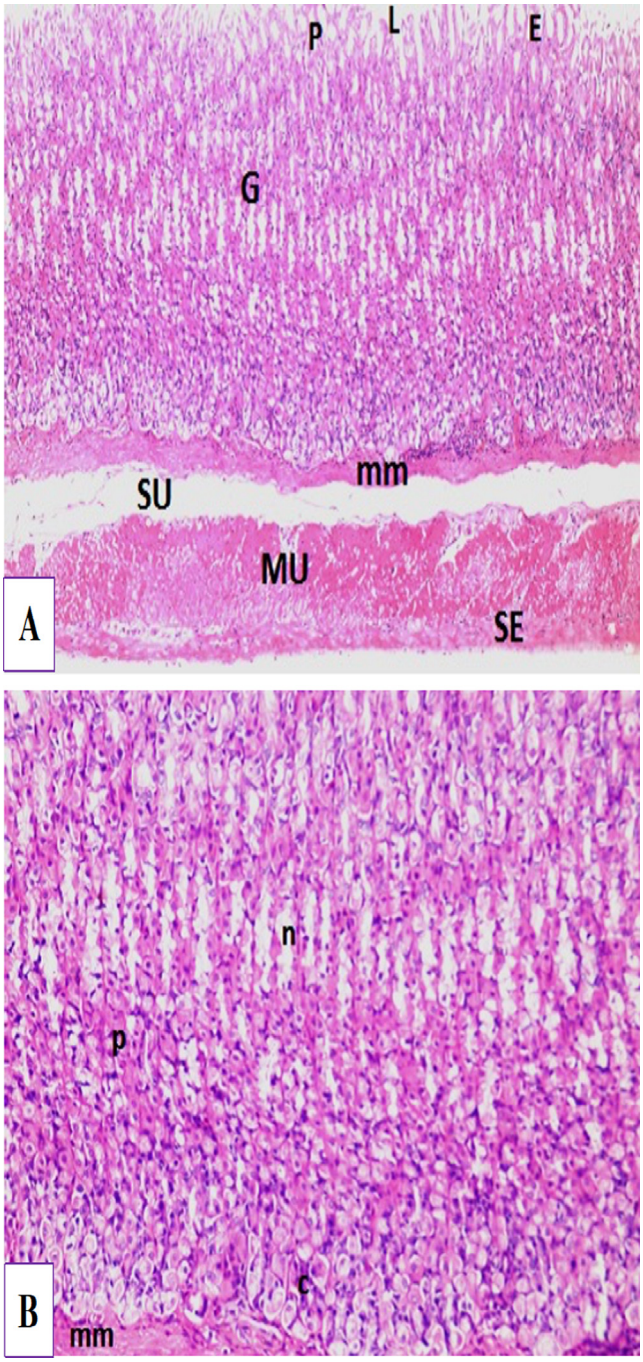


Fig. 1. A photomicrograph of stomach wall of a control rat showing normal regularly arranged mucosal gastric gland (G) arranged perpendicular to the muscularis mucosa (mm), gastric pits (P), submucosa (SU), musculosa (MU) and serosa (SE). Notice the gastric lumen (L) close to the surface epithelium (E) (A). Higher magnification (B) showing most of the bulk of stomach mucosa is full of secretory cells of the gastric glands which seem to form cord-like bands of cells perpendicular to the muscularis mucosa (mm). Primarily parietal cells (p) which are rounded cells with central deeply stained nuclei and eosinophilic cytoplasm. The chief cells (C) are pyramidal in shape with basal nucleus and basophilic cytoplasm and mucous neck cells (n) **(Hx. & E. Ax100 & Bx400)**.

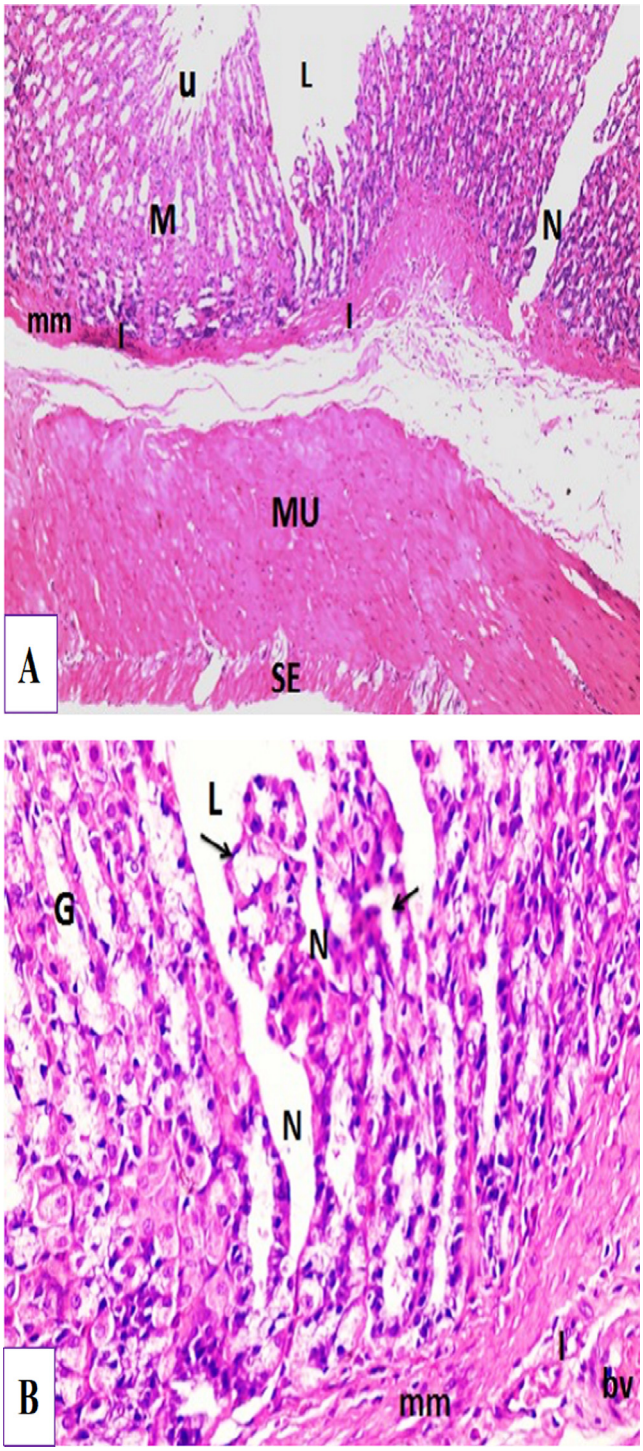


Fig. 2. A photomicrograph of stomach wall of CPX treated rat showing extensive disruption to the surface epithelium (N) of gastric mucosa (M) reaching the muscularis mucosa (mm) which is exposed to gastric lumen (L) with edema and leucocytes infiltration (I) of the muscularis mucosa and sub-mucosal layer (SU). Normal musculosa (MU) and serosa (SE) could be realized (A). Higher magnification (B) showing extensive epithelial sloughing (N), pyknotic nuclei (arrows). Dilated submucosal blood vessel (bv) with a surrounding cellular infiltrate (I) can be seen. The surface stomach mucosa having striking vacuolations of the gastric gland cells (G). Unbroken muscularis mucosa (mm) exposed to gastric lumen (L) is noticed. **(Hx. & E. Ax100 & Bx400)**.

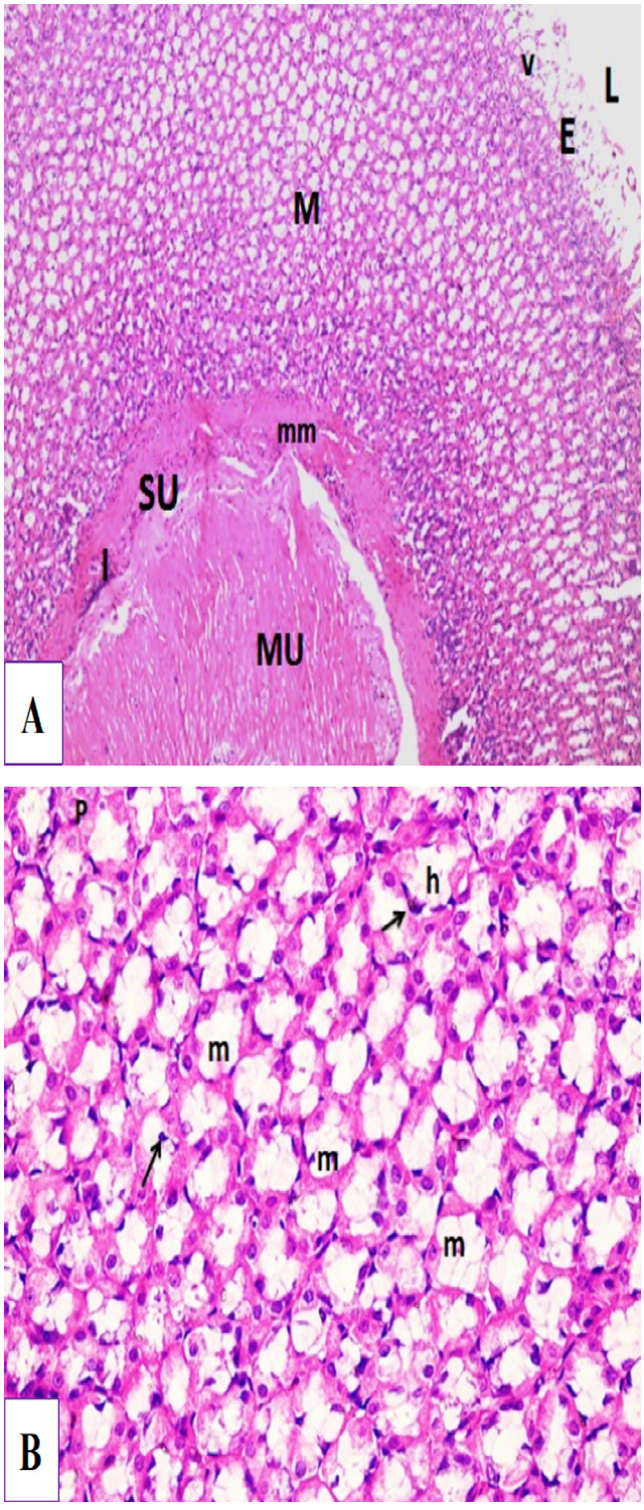


Fig. 3. A photomicrograph of fundus of CPX treated stomach rat showing surface epithelium (E) with vacuolations (v) close to the gastric lumen (L), giving a honey combing appearance of gastric mucosal glands (M). Inflammatory cell infiltration (I) in muscularis mucosa (mm) of the mucosal layer and submucosal edema (SU) close to musculosa (MU) (A). Higher magnification (B) showing severely widened cystic glandular structures, hydropic alterations in some gastric cells (h) though other shows small pyknotic nuclei (arrows). Vacuolated mucus cells (m) are aggregated giving rosette appearance with intact parietal (p) cells scattered in-between **(Hx. & E. Ax100 & Bx400)**.

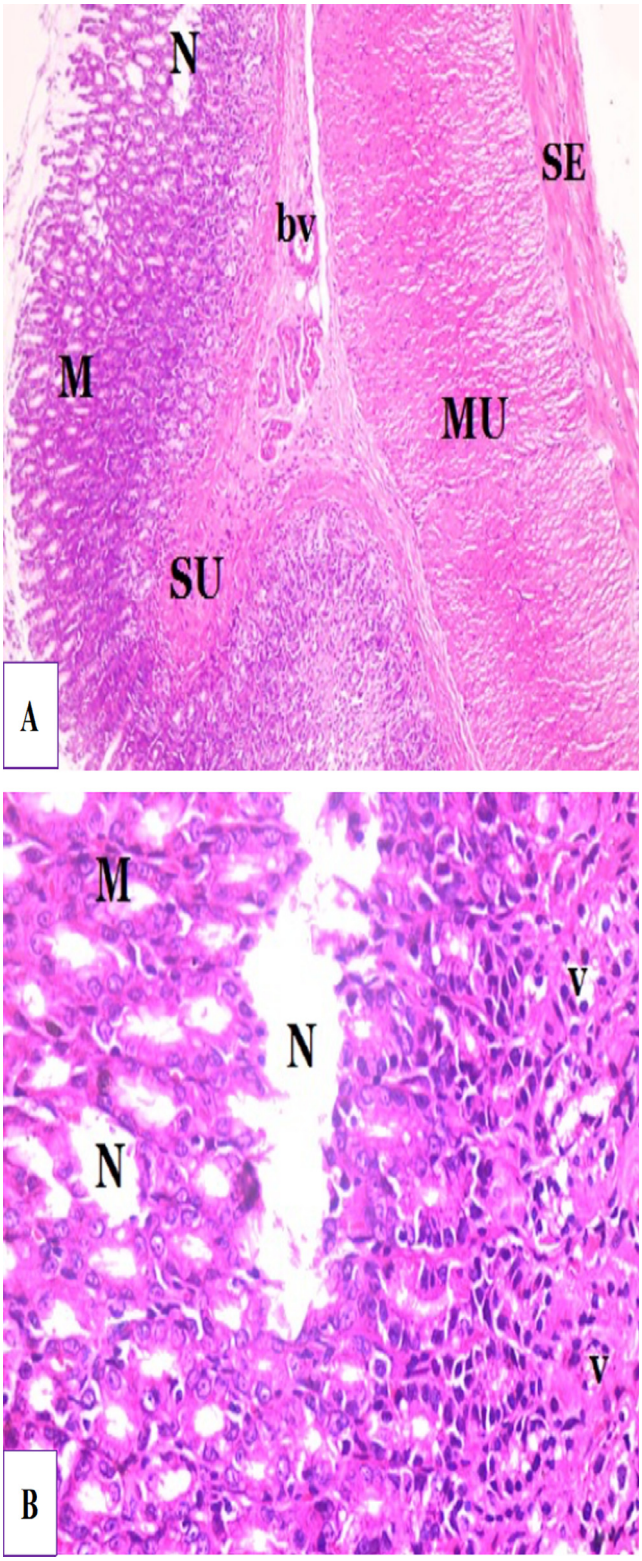


Fig. 4. A photomicrograph of rat gastric wall of CPX withdrawal group (group III) showing stomach mucosa (M) with limited necrotic areas (N). It also reveals submucosal layer (SU) with congested blood vessels (bv). This section also shows normal musculosa (MU) and serosa (SE) (A). Higher magnification (B) showing dilated gastric mucosal glands (M) with limited areas of necrosis (N). It also exhibits nesting of parietal cells with vacuolated cytoplasm (v) **(Hx. & E. Ax100 & Bx400)**.

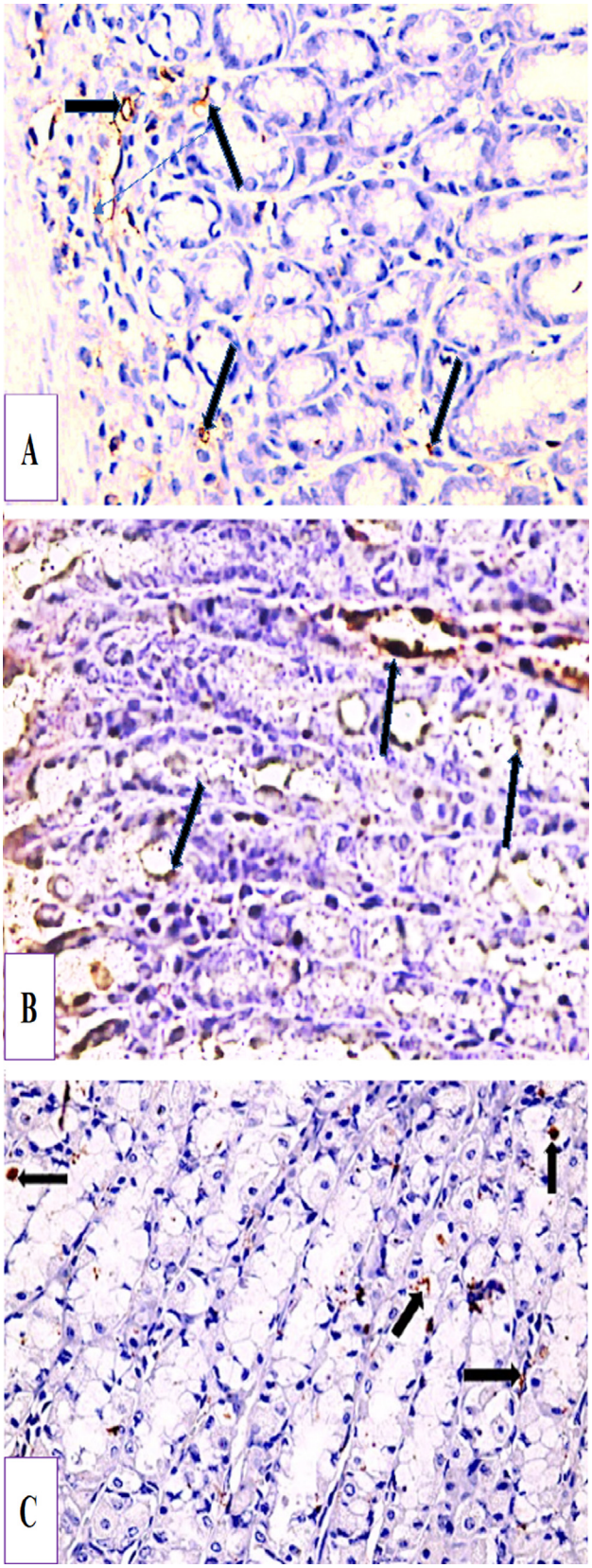


Fig. 5. A photomicrograph of stomach sections presenting mild positive reaction for TNFα expression in the control rat (Group I; A), severe positive reaction for TNFα expression in the rats treated with CPX (Group II; B) and moderate positive reaction for TNFα expression in the withdrawal group **(Group III; C)**. **(TNFα immunostaining × 400)**.

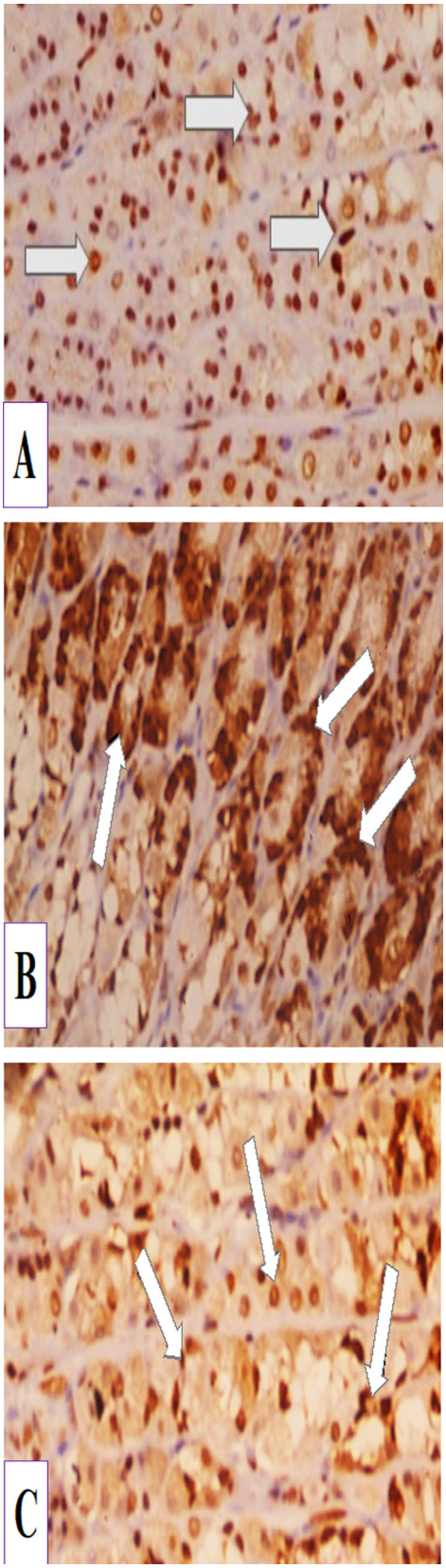


Fig. 6. A photomicrograph of stomach sections presenting mild positive reaction for TNFα expression in the control rat (Group I; A), strong positive reaction for TNFα expression in the rats treated with CPX (Group II; B) and moderate positive reaction for TNFα expression in the withdrawal group (Group III; C). **(PCNA immunostaining × 400)**.

3.2. Scanning electron microscopic results

Scanning electron microscopy of control rat gastric mucosa (Group I) exhibited healthy epithelial cells with gastric glands and pits (Fig. 7). CPX treated rat gastric mucosa (Group II) showed damaged epithelial cells (erosions) and widened gastric pits (Fig. 8A). Other areas showed a number of separate stomata in the basement membrane, through which young mucous epithelial cells sprouted out of the gastric glands' lamina propria. Necrotic gastric epithelium and polymerized mucus could be seen. Moreover, connective tissue meshwork of gastric gland without lining epithelial cells could be noticed (Fig. 8B). Honey combing appearance of gastric mucosa was also seen in some areas with flattened gastric glands widened gastric pits (Fig. 8C). Higher magnification showed nearby intestinalized regions revealing a plush microvillous with striated border occasionally disturbed by apical apertures of goblet cells. Widened gastric pits could be noticed with apparent H.pylori cocci (Fig. 8D). Rat gastric mucosa of the withdrawal group (Group III) showed interaction between H. pylori cocci and gastric epithelial cells in the form of intestinalized regions with plush microvilli and striated border periodically interjected by apical apertures of goblet cells (Fig. 9A). In some other areas, the basement membrane was noticed with the gastric gland cells migrating out of the lamina propria and bundles of microfilaments lying on its outer surface. Some deep gastric pits were noticed (Fig. 9B).

It also revealed partial recovery in the form of healthy epithelial cells, gastric glands with intervening gastric pits. However, Few H. pylori cocci could be seen in the depth of some gastric pits (Fig. 9C).

3.3. Morphometric and statistical results

See Table 3A, Table 3B, Table 3C, and Histogram 1–3.

4. Discussion

The current research studied the effect of CPX on the normal organization of gastric wall of adult rats and the possible detection of H. Pylori both histologically and serologically. The stomach of the control rats was lined by epithelial cell layers containing several gastric pits lined by mucous cells, and continuous with the tube-like glands occupied by numerous cell lines manufacturing mucus, acid, pepsinogen, and numerous hormones. Karam et al. (2003) reported that these cell lines originate from multiplying epithelial stem cells. Cells with basally basophilic cytoplasm and basal nuclei are the chief cells. These are accumulated in the lower areas of the glands. Cells with obvious eosinophilic cytoplasm and centrally located nuclei (fried egg appearance) are the parietal cells. These are focused in the middle to upper areas of the glands. The glands are separated by tinny threads of lamina propria containing the denser, flattened or irregular nuclei of fibroblasts, capillary endothelium, and numerous cells of the immune system.

Microstructural examination of gastric sections of CPX rats revealed that the CPX-induced zones of focal erosion (scattered damage) in the stomach lining that became eroded and ulcerated to the extent that the muscularis mucosae became open to the gastric lumen, widespread leucocyte infiltration and edema of the sub-mucosal stratum, congestion and dilatation of the gastric sub-mucosal blood vessels. These results were parallel to the outcomes of (Barnett et al., 2000) who detected considerable necrosis, epithelial sloughing, and shallow ulcers following drug management.

Morini and Grandi (2010) and Lim et al. (2019) reported that Stomach mucosa has numerous defensive mechanisms that permit

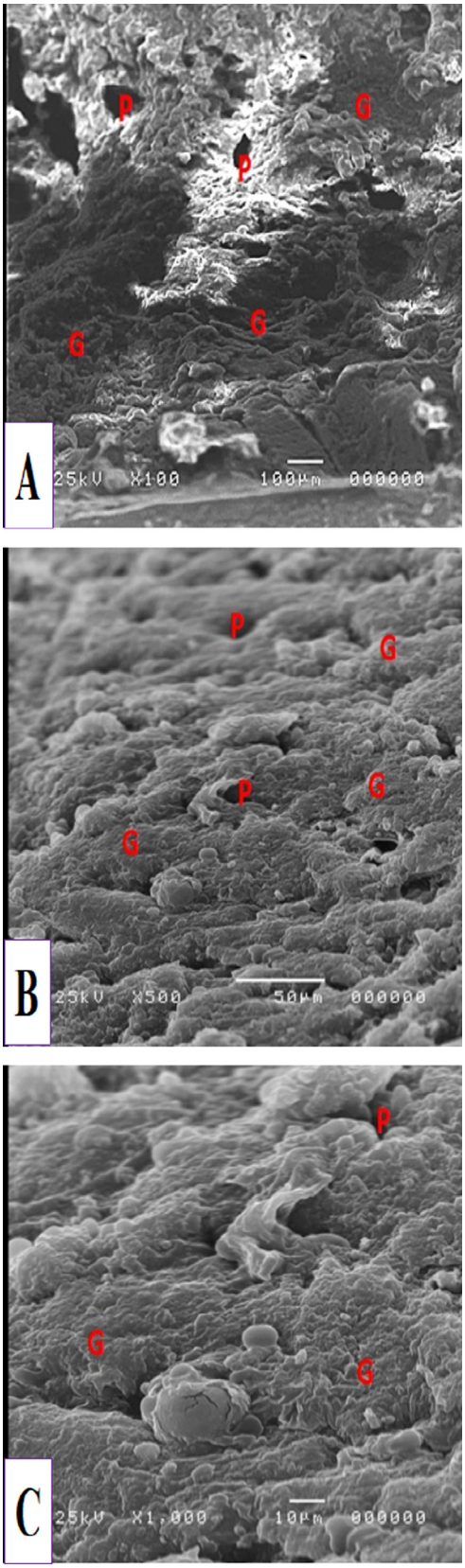


Fig. 7. A scanning electron photomicrograph of a control rat gastric mucosa (Group I) showing healthy epithelial cells, gastric glands (G) with intervening gastric pits (P). **(SEM Ax100, Bx500 & Cx1000)**.

the mucosa to tolerate repeated exposure to possibly destructive factors. The discrepancy between protective and forceful factors is at the heart of the creation of lesions or ulcerations of the stomach mucosa. The dissimilarity between an erosion and ulceration is that the former is limited to the mucosa, while ulceration infiltrates to the muscularis mucosae. They also clarified how to measure gastric mucosal injuries by microscopic analysis of the stomach via light and by scanning electron microscopy.

Anto et al. (2001) also indicated that, gastritis or inflammation of gastric mucosa is not a distinct illness, but relatively a collection of panics, frequently accountable for inflammatory alterations in gastric mucosa. Nonetheless, they fluctuate in their clinical features, histological expressions and causal mechanisms. Furthermore, kim et al. (2011) reported that the progress of the gastric induced mucosal injuries might furthermore be facilitated through generation of oxygen free radicles. These results were similar to that of Palacios-Espinosa et al. (2014) and Shalaby et al. (2016) who specified that in occasion of stomach ulceration, the neighboring blood vessels looked expanded and congested. In this study, there was inflammatory cellular infiltration in the lamina propria of the gastric mucosa, goes equivalent to the results recognized by Boushra et al. (2019) who stated that, there was dense cellular infiltration detected in the ulcer beds of the fundic lamina propria. They described the infiltration with mononuclear cells due to the effect of NSAIDs on the integrity of intercellular junctions and on suppressing the manufacture of the defensive mucus coat. Accordingly, the mucosa will be damaged by the acid and proteolytic enzymes associated with consequent H. pylori attack of the epithelium. These bacteria were chemotactic for WBCs, lymphocytes and macrophages. Besides, they explained that, polymorphonuclear leucocyte infiltration might be due to H. pylori infection which is associated with drug-induced ulcers. Wallace (2001) said that the existence of acid in the lumen of the stomach might not be a key element in the pathogenesis of drug-induced gastroenteropathy. It could create a significant support to the chronicity of such injuries and to bleeding by weakening the restoration procedure, interfering with hemostasis and deactivating numerous growth elements that are vital in mucosal defense and restoration.

In this study, Gastric slices from CPX group displayed moderate positive reaction for TNFα expression as matched to faint positive reaction in the control and CPX-withdrawal groups. Positive findings of the immunohistochemical staining are displayed in brown color by light microscope. This agrees with khoder et al. (2019) who exhibited that experimental animals developed augmented manufacture of mucus, reduced making of pepsinogen and augmented ghrelin-secreting cells. Apoptosis and necrosis are double distinguishing varieties of cell demise, which Ramseyer and Garvin (2013) also reported that TNFα is a type of pro-inflammatory cytokine that rises in chronic inflammatory conditions and mediates organ injury by exciting immune cell infiltration and cell death.

Bimczok et al. (2013) and Gonciarz et al. (2019) also reported that H. pylori infection encourages cell apoptosis in concurrence with augmented oxidative stress. Raised apoptosis keeps against injurious inflammation and neoplasia; nevertheless, it diminishes cell integrity in addition to up-regulation of cell immigration and multiplication in response to damage. They also discovered that epithelial cells experiencing apoptosis as a reaction to live H. pylori were not eradicated by human gastric mononuclear phagocytes, however they were under the effect of TNF-α, produced by macrophages and extremely displayed in H. pylori-infected gastric mucosa compared to control cells. Ernst et al. (2006) and Amieva and El–Omar (2008) reported that in humans, epithelial cell mor-

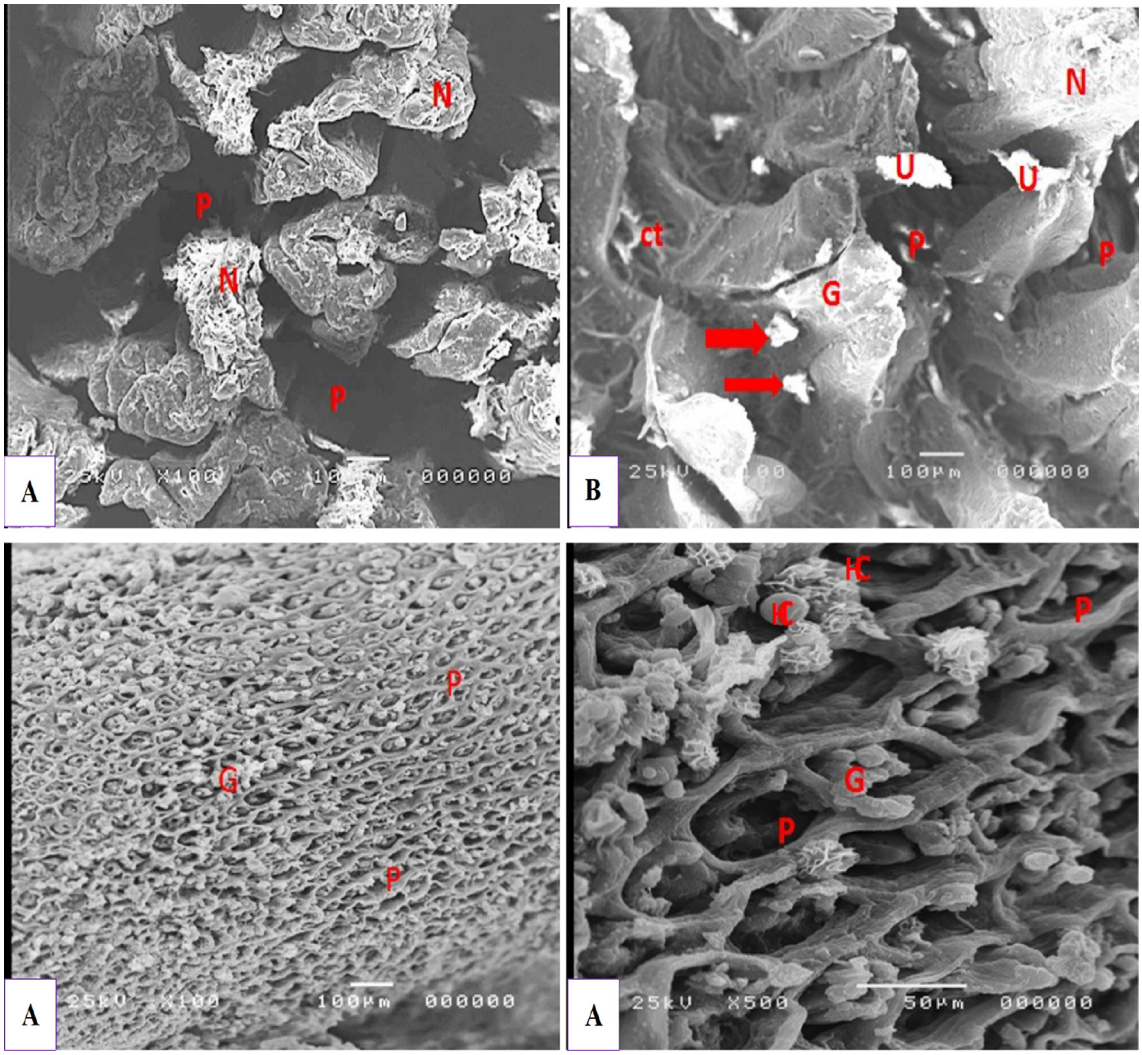


Fig. 8. A scanning electron photomicrograph of gastric mucosa of CPX treated rat (Group II) showing damaged epithelial cells with erosions and ulceration (N) and widened gastric pits (P) (A). Other areas show a number of separate holes in the basement membrane, over which Young mucous epithelial cells (red arrows) sprouting out of the gastric glands lamina propria (G). Necrotic gastric epithelium (N) and polymerized mucus (U) can be seen. Moreover, connective tissue meshwork of gastric gland without lining epithelial cells (ct) could be noticed (B). Honey combing appearance of gastric mucosa is also seen in some areas with flattened gastric glands (G) and widened gastric pits (P) (C). Higher magnification shows a nearby intestinalized region revealing a plush microvillous (m) with striated border occasionally interjected by apical apertures of goblet cells (red arrows) with surrounding widened gastric pits (P). Few H. pylori cocci (H) could be seen (Fig. 7D). **(SEM A, B & Cx100; Dx500)**.

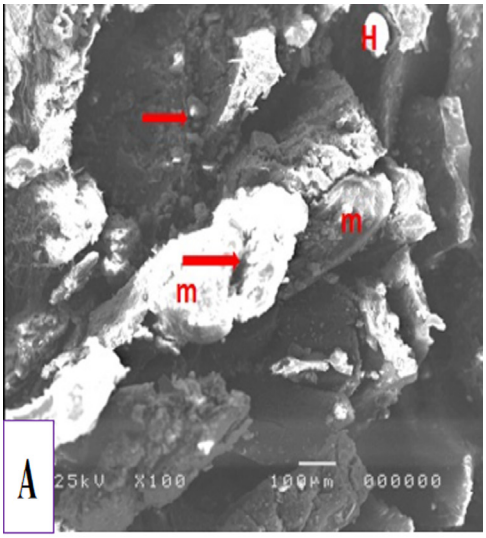
phology was modified by H. pylori infection which also produced interruption of tight connections, stimulation of cytokine manufacture, epithelial cell proliferation, and rise in cell death via apoptosis.

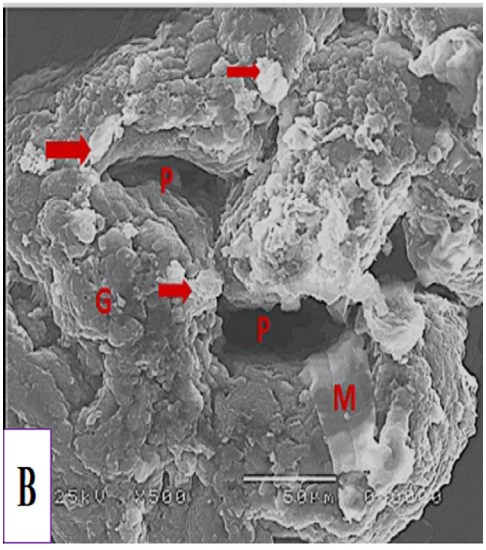
In the current study, scanning electron microscopy of gastric mucosa of CPX group exhibited damaged epithelial cells, erosions with widened gastric pits. Zaki & Mohamed, 2014 also reported that ulcerations at higher magnification presented the connective tissue framework of stomach gland corion restituted of normal epithelial cells but showing young traveling cells emergent out from glandular pit. The fundus of the stomach presented gastric ulcer spreading up to the muscularis mucosa. The mucosal cells adjoining the round gastric ulcer are the body's challenge to heal the damage by wrapping the ulcer. Round these cells the coarse texture of healthy gastric mucosa could be seen.

A scanning electron microscope photomicrograph of other areas of the stomach wall of CPX treated rats revealed mucus, widened gastric pits with Interaction between H. pylori cocci and gastric epithelial cells. Moreover, intestinalized region with a plush microvillus, striated border occasionally disturbed by apical stomata of goblet cells was noticed. This agrees with Winborn and Weser (1983) who registered features consistent with those described in chronic gastritis; represented by amplification of the "cobblestone relief" look of the luminal surface of the stomach and the existence of several little, short, bulging microvilli projecting from the free edge of the surface mucous cells to the stomach lumen.

Furthermore, the scanning electron microscopy of the gastric mucosa of rats of the CPX group recorded widened gastric pits,

Fig. 9. A scanning electron photomicrograph of gastric wall of a rat in the withdrawal group (Group III) showing Interaction between H. pylori cocci (H) and gastric epithelial cells. It also shows a nearby intestinalized region revealing a plush microvillous (m) with striated border periodically interjected by apical opertures of goblet cells (red arrows) (A). In some areas, the basement membrane (Thick red arrows) is noticed with the gastric gland cells (G) migrating out of the lamina propria with a bundle of microfilament (M) lying on its outer surface. Deep gastric pits (P) are noticed (B). However, in other areas, healthy epithelial cells, gastric glands (G) with intervening gastric pits (P) can be seen. Few H. pylori cocci (H) could be seen in the depth of gastric pit (C). **(SEM Ax100, Bx500 & Cx1000)**.





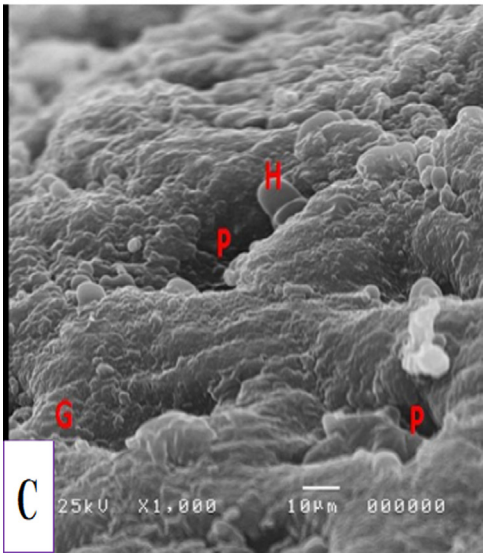


Table 1
TNFα percentage area expression.

TNFα percentage area Parameters	Control	CPX	CPX-Withdrawal
Range	8.29–18.12	32.68–46.71	10.33–23.65
Mean ± SD	12.289 ± 4.185	38.526 ± 4.199	15.735 ± 4.373
F test	**119.073**		
P value	**<0.001****		
Control & Experimental	**Control & Withdrawal**		**Experimental & Withdrawal**
< 0.001**	**0.0886**		**<0.001****

Results are expressed as mean ± SD.
** $P < 0.001$ means highly significant variances.

Table 2
PCNA percentage area expression.

PCNA percentage area Parameters	Control	CPX	CPX-Withdrawal
Range	9.31–21.92	37.26–47.64	11.26–21.28
Mean ± SD	13.168 ± 4.243	41.545 ± 3.505	16.516 ± 3.557
F test	**168.006**		
P value	**<0.001****		
Control & CPX	**Control & CPX-Withdrawal**		**CPX & CPX-Withdrawal**
< 0.001**	**0.0719**		**<0.001****

Results are expressed as mean ± SD.
** $P < 0.001$ means highly significant variances.

Table 3A
H. pylori IgM. **Laboratory results of serum level of H. pylori IgM.** Statistical analysis of serum level of H. pylori **IgM** results showed highly significant (P < 0.05).

IgM Parameters	Control	CPX	CPX-Withdrawal
Range	0.33–0.85	0.31–0.38	0.6–0.7
Mean ± SD	0.62 ± 0.20	0.34 ± 0.03	0.63 ± 0.04
F test	**9.424**		
P value	**0.003***		
Control & Experimental	**Control & Withdrawal**		**Experimental & Withdrawal**
0.003*	**0.876**		**0.002***

* $P < 0.05$ means significant variances.

Table 3B
H. pylori IgA. **Laboratory results of serum level of H. pylori IgA.** Statistical analysis of serum level of H. pylori **IgA** results showed highly significant (P < 0.05)

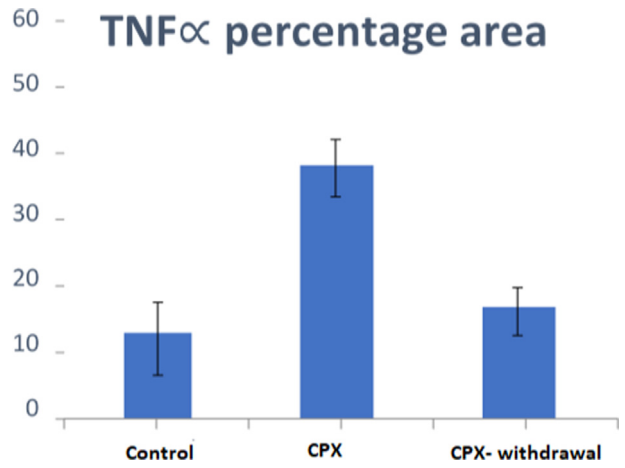
IgA Parameters	Control	CPX	CPX-Withdrawal
Range	0.22–0.85	0.29–0.39	0.59–0.7
Mean ± SD	0.63 ± 0.26	0.33 ± 0.04	0.64 ± 0.05
F test	**6.792**		
P value	**0.011***		
Control & Experimental	**Control & Withdrawal**		**Experimental & Withdrawal**
0.008*	**0.984**		**0.007***

* $P < 0.05$ means significant variances.

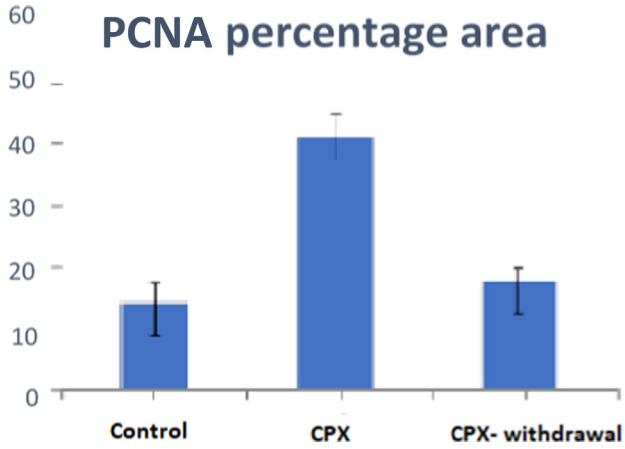
Table 3C
H. pylori IgG. **Laboratory results of serum level of H. pylori IgG.** Statistical analysis of serum level of H. pylori **IgG** results showed highly significant (P < 0.05).

IgG Parameters	Control	CPX	CPX-Withdrawal
Range	0.5–1	0.3–0.45	0.39–0.7
Mean ± SD	0.71 ± 0.21	0.36 ± 0.06	0.58 ± 0.11
F test	**7.659**		
P value	**0.007***		
Control & CPX	**Control & Withdrawal**		**CPX & CPX-Withdrawal**
0.002*	**0.183**		**0.030***

* $P < 0.05$ means significant variances.



Histogram 1. TNF ∝ percentage area in all groups.

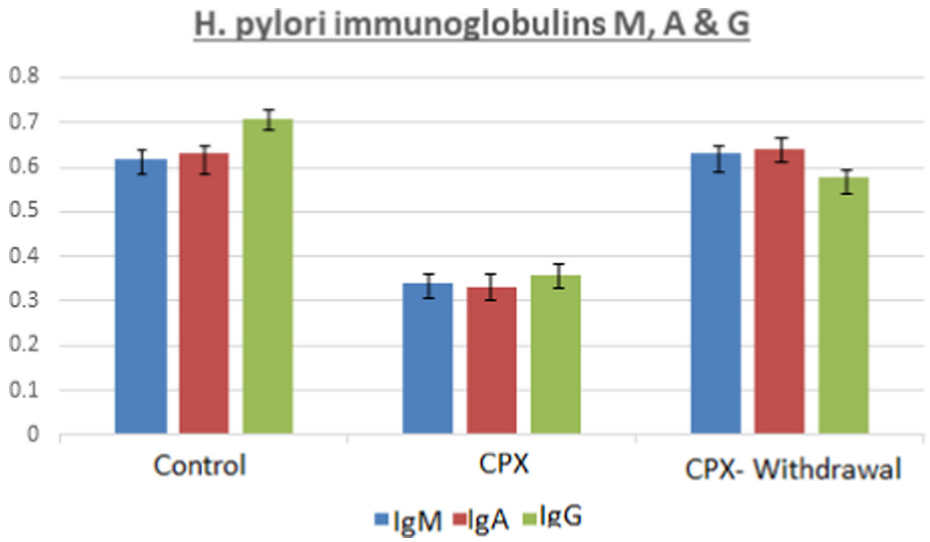


Histogram 2. PCNA percentage area in all groups.

the basement membrane showed a number of separate openings through which young mucous epithelial cells sprouting out from gastric glands cells traveling out of the lamina propria with a bundle of microfilament extending on the external surface of the basement membrane. This agrees with WU et al., 1999 who recorded cells traveling out through basement membrane holes of the human gastric lamina propria, after the damage of the mucosal lining. Lamina propria cells traveling out of cultured shed gastric mucosal sections were characterized phenotypically and functionally. Such cells would be appropriate for researching their connections with epithelial cells and moreover with H. pylori and its products. H. pylori or its products would be accomplished by achievement entrance into the visible lamina propria, through basement membrane holes. Such entrance to the lamina propria might happen earlier than epithelial continuity and barrier function became respectable by immigration of neighboring viable cells. Their work suggested that lamina propria cells (lymphocytes macrophages and myofibroblasts) got through a culture of shed gastric mucosal models would be appropriate for recognition of their interactions with H. pylori and its products. Enroth et al. (1999) and Kusters et al. (2006) indicated that H. pylori is a gram-negative bacterium, measuring 2 to 4 μm in length and 0.5–1 μm in width. Though frequently spiral-shaped, the bacterium could seem like a rod, while coccoid forms look after extended in vitro culture or antibiotic therapy which was recognized in the current study by scanning electron microscopy. These coccoids could not be cultured in vitro and are supposed to signify unviable cells, while it had been proposed that coccoid forms might elucidate a viable, non-culturable form.

It was documented in this research that CPX treated rats exhibited insignificant deviation of serum anti H. Pylori immunoglobulins compared to the control and CPX- withdrawal groups. Granberg et al. (1993) mentioned that investigation for IgG antibodies, is a respectable and dependable test for the recognition of antibodies to H. pylori and as a clue of H. pylori infection. The determination of IgA antibodies might be used as a test that supplements the IgG antibody assessment. This also agrees with Shao et al. (2003) who determined the frequency of H. pylori infection by the serum anti-H. pylori immunoglobulin G (IgG) and IgA antibody reactions, and the significance of clinical presentations in the identification of H. pylori contamination in patients with gastric atrophy, intestinal metaplasia, and dysplasia by enzyme-linked immune-adsorbent assays (ELISA).

Silva et al., 2001 also specified that the recognition of H. pylori in the gastric epithelium and its association with the peptic disease



Histogram 3. Serum level of H. pylori immunoglobulins M, A & G in all groups.

lead to a notable alteration in the controlling of peptic ulcers. It is a universal accord that suppression of the bacterium is the cornerstone of peptic ulcer therapy. The low rates of suppression observed in mono and dual treatments encouraged the usage of double or three antibiotics and a proton pump inhibitor. Dresner et al., 1996 reported that CPX once combined with omeprazole and bismuth is effective for the eradication of H pylori. Dore et al., 2012 also displayed that Ciprofloxacin-based therapy treated 65% of patients as an alternate to bismuth established quadruple treatment. This agrees with our serological findings of significant reduction of serum level of H. Pylori IgM, IgA, and IgG in CPX group.

5. Conclusion

It could be concluded that prolonged oral CPX administration to albino rats changes gastric mucosal physiology and architecture but never aggravates infection with H. Pylori. It is also recommended to accurately track patients under treatment concomitantly to avoid any possible side effects of gastric ulcerations and erosions.

Compliance with ethics guidelines

This research was approved by research ethics committee, Tanta Faculty of Medicine; approval code number: 34441/2/21.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Altwaijry, N., El-Masry, T., Alotaibi, B., Tousson, E., Alodhayani, A., El-Morshedy, K., Elmaghed, N., Sayed, A., Saleh, A., 2021. Potential therapeutic effects of avenanthramide-C against lung toxicity caused by silver nanoparticles injection in rats. Pak. J. Pharm. Sci. 34(1) (Suppl) 337–343.

AL-Wajeeh, N., Hajerezaie, M., Ali, H., Abdulla, M., 2017. The gastro protective effects of Cibotium barometz hair on ethanol-induced gastric ulcer in Sprague-Dawley rats. BMC Veterinary Res. 13, Article number: 27 (2016).

Amieva, M.R., El–Omar, E.M., 2008. Host-bacterial interactions in *Helicobacter pylori* infection. Gastroenterology 134 (1), 306–323.

Antos, D., Enders, G., Rieder, G., Stolte, M., Bayerdörffer, E., Hatz, R.A., 2001. Inducible nitric oxide synthase expression before and after eradication of Helicobacter pylori in different forms of gastritis. FEMS Immunol. Med. Microbiol. 30, 127–131.

Bancroft, J.D., Gamble, M., 2008. Theory and Practice of Histological Techniques. Churchill Livingstone, Elsevier, London.

Barnett, K., Bell, C.J., McKnight, W., Dicay, M., Sharkey, K.A., Wallace, J.L., 2000. Role of cyclooxygenase-2 in modulating gastric acid secretion in the normal and inflamed rat stomach. Am. J. Physiol. Gastrointest. Liver Physiol. 279 (6), G1292–G1297.

Bimczok, D., Smythies, L.E., Waites, K.B., Grams, J.M., Stahl, R.D., Mannon, P.J., 2013. *H. pylori* infection inhibits phagocyte clearance of apoptotic gastric epithelial cells. J. Immunol. 15, 6626–6634.

Boushra, A., Elsayed, A., Ibrahim, N., Abdelwahed, M., Ahmed, E., 2019. A comparative study on the possible protective effect of esomeprazole, spirulina, wheatgrass on indomethacin-induced gastric ulcer in male albino rats. Mol. Biol. Rep. 46(5), 4843–4860. doi: 10.1007/s11033-019-04933-1. Epub 2019 Jul 11. https://pubmed.ncbi.nlm.nih.gov/31297714/#affiliation-4.

Buchwalow, I.B., Böcker, W., 2010. Immunohistochemistry: Basics and Methods. Springer, Heidelberg, Dordecht, London, New York, pp. 31–39.

Butt, J., Epplein, M., 2019. Helicobacter pylori and colorectal cancer—A bacterium going abroad? Plos pathogens.Published: August 8, 2019. https://doi.org/10.1371/journal.ppat.1007861.

Calabrese, F., Carturan, E., Chimenti, C., Pieroni, M., Agostini, C., Angelini, A., Crosato, M., Valente, M., Boffa, G.M., Frustaci, A., Thiene, G., 2004. Overexpression of tumor necrosis factor (TNF) α and TNFα receptor I in human viral myocarditis: Clinicopathologic correlations. Modern Pathol. 17 (9), 1108–1118.

Carman, R.J., Woodburn, M.A., 2001. Effects of low levels of ciprofloxacin on a chemostat model of the human colonic microflora. Regul. Toxicol. Pharm. 33 (3), 276–284. https://doi.org/10.1006/rtph.2001.1473. Chem, 25(146), pp: 599-612.

Dawson, B., Trapp, R., 2004. Basic & Clinical Biostatistics: Fourth Edition. Chapter 3. McGraw Hill Professional. ISBN: 9780071781329.

Debraekeleer, A., Remaut, H., 2018. Future perspective for potential Helicobacter pylori eradication therapies. Future Microbiol. 13 (6), 671–687.

Dore, M., Tadeu, V., Are, B., Mura, I., Fanciulli, G., Massarelli, G., Piana, A., 2012. Efficacy of a "Rescue" Ciprofloxacin-Based Regimen for Eradication of Helicobacter pylori Infection after Treatment Failures. Gastroenterol Res. Pract. 2012, 484591. doi: 10.1155/2012/484591. Epub 2012 May 14.

Dresner, D., Coyle, W., Nemec, R., Peterson, R., Duntemann, T., Lawson, J.M., 1996. Efficacy of ciprofloxacin in the eradication of Helicobacter pylori. South Med J. 89(8), 775–778. doi: 10.1097/00007611-199608000-00004.

El-Masry, T., Al-Shaalan, N., Tousson, E., Buabeid, M., Al-Ghadeer, A., 2020. Potential therapy of vitamin B17 against Ehrlich solid tumor induced changes in Interferon gamma, Nuclear factor kappa B, DNA fragmentation, p53, Bcl2, survivin, VEGF and TNF-α Expressions in mice. Pak. J. Pharm. Sci. 33 (1), 393–401.

Emam, M., Moustafa, P., Elkhateeb, A., Hussein, S., Marzouk, M., Abd El-Rahman, S., Abdel-Hameed, E., Abdel-Rahman, R., 2021. Dobera glabra (Forssk.) Poir. (Salvadoraceae); phenolic constituents of the aqueous leaves extract and evaluation of its anti-inflammatory, analgesic activities. Heliyon 7, e06205.

Enroth, H., Wreiber, K., Rigo, R., Risberg, D., Uribe, A., Engstrand, L., 1999. In vitro aging of Helicobacter pylori: changes in morphology, intracellular composition and surface properties. Helicobacter 4(1), 7–16. doi: 10.1046/j.1523-5378.1999.09034.x

Ernst, P.B., Peura, D.A., Crove, S.E., 2006. The translation of Helicobacter pylori basic research to patient care. Gastroenterology 130, 188–206.

Eskandari, M., Ghalyanchi Langeroudi, A., Zeighami, H., Rostami, A., Fontana, G., Licciardi, M., Mansueto, S., Schillaci, D., Giammona, G., 2001. Amoxicillin-loaded polyethylcyanoacrylate nanoparticles: Influence of PEG coating on the particle size, drug release rate, and phagocytic uptake. Biomaterials 22, 2857–2865.

Gonciarz, W., Krupa, A., Hinc, K., Obuchowski, M., Moran, A., Gajewski, A., Chmiela, M., 2019. The effect of Helicobacter pylori infection and different H. pylori components on the proliferation and apoptosis of gastric epithelial cells and fibroblasts. Plos one. Published: August 7, 2019.

Gotoh, A., Akamatsu, T., Shimizu, T., 2002. Additive effect of pronase on the efficacy of eradication therapy against Helicobacter pylori. Helicobacter 7 (3), 183–191.

Granberg, C., Mansikka, A., Lehtonen, O.P., Kujari, H., Grönfors, R., Nurmi, H., Räihä, I., Ståhlberg, M.R., Leino, R., 1993. Diagnosis of Helicobacter pylori infection by using pyloriset EIA-G and EIA-A for detection of serum immunoglobulin G (IgG) and IgA antibodies. J. Clin. Microbiol. 31(6), 1450–1453.

Hajrezaie, M., Golbabapour, S., Hassandarvish, P., Gwaram, N.S., Hadi, A.H.A., 2012. Acute toxicity and gastro protection studies of a new Schiff base derived Copper (II) Complex against ethanol induced acute gastric lesions in rats. PLoS ONE 7, (12) e51537.

Hasan, A., Thulfiqar, F., Mutar, Tousson, E., Felemban, S., 2021. Therapeutic effects of *Turnera diffusa* extract against amitriptyline-induced toxic hepatic inflammation. OnLine J. Biol. Sci. 2021, 21(2): 395.408 DOI: 10.3844/ojbsci.2021.395.408 https://doi.org/10.1371/journal.pone.0220636

Karam, S.M., Straiton, T., Hassan, W.M., Leblond, C.P., 2003. Defining epithelial cell progenitors in the human oxyntic mucosa. Stem Cells. 21, 322–336.

Khaki, A., Heidari, M., Novin, M.G., Khaki, A., 2008. Adverse effects of ciprofloxacin on testis apoptosis and sperm parameters in rats. Iranian J. Reprod. Med. 6 (2), 71–76.

Khoder, G., Al-Yassir, F., Al Menhali, A., Saseedharan, P., Sugathan, S., Tomasetto, C., Karam, S., 2019. Probiotics upregulate trefoil factors and downregulate pepsinogen in the mouse stomach. Int. J. Mol. Sci. 20 (16), 3901.

Kim, J.H., Kim, B.W., Kwon, H.J., Nam, S.W., 2011. Curative effect of selenium against indomethacin-induced gastric ulcers in rats. J. Microbiol. Biotechnol. 21, 400–404.

Kusters, J., van Vliet, A., Kuipers, E., 2006. Pathogenesis of *Helicobacter pylori* Infection. Clin. Microbiol. Rev. 19(3), 449–490. doi: 10.1128/CMR.00054-05.

Lim, J.M., HyunSong, C.H., Park, S.J., Park, D.C., Jung, G.W., Cho, H.R., Bashir, M., Kwang, Ku.S., Choi, J.S., 2019. Protective effects of triple fermented barley extract (FBe) on indomethacin-induced gastric mucosal damage in rats. BMC Complement. Alternative Med. 19, Article number: 49.

Michalak, K., Włodarczyk, A., Włodarczyk, M., Sobolewska, J., Woźniak, P., Sobolewski, B., 2017. Treatment of the Fluoroquinolone-Associated Disability: The Pathobiochemical Implications. Xenobiotics, Oxidative Stress, and Antioxidants. Volume 2017|ArticleID 8023935 | https://doi.org/10.1155/2017/8023935

Mori, N., Sato, H., Hayashibara, T., Senba, M., Geleziunas, R., Wada, A., Hirayama, T., Yamamoto, N., 2003. Helicobacter pylori induces matrix metalloproteinase-9through activation of nuclear factor κB. Gastroenterology 124 (4), 983–992.

Morini, G., Grandi, D., 2010. Methods to Measure Gastric Mucosal Lesions in the Rat. Curr. Protocols Toxicol. 43(1), 21.2.1-21.2.15. https://currentprotocols.onlinelibrary.wiley.com/doi/full/10.1002/0471140856.tx2102s43

Palacios-Espinosa, J.F., Arroyo-Garcia, O., Garcia-Valencia, G., Linares, E., Bye, R., Romero, I., 2014. Evidence of the anti-Helicobacter Pylori, gastroprotective and anti-inflammatory activities of Cuphea aequipetala infusion. J. Ethnopharmacol. 151, 990–998.

Ramseyer, V., Garvin, J., 2013. Tumor Necrosis Factor alpha: regulation of renal function and blood pressure. AJP Renal Physiol. 304 (10), F1231–F1242.
Reagan-Shaw, S., Nihal, M., Ahmad, N., 2008. Dose translation from animal to human studies revisited. FASEB J. 22 (3), 659–661.
Schumann, C., Triantafilou, K., Rasche, F.M., Angelika, Möricke, A., Vogt, K., Triantafilou, M., Hahn, P., Schneider, E., Lepper, P., 2006. Serum antibody positivity for distinct Helicobacter pylori antigens in benign and malignant gastroduodenal disease. Int. J. Med. Microbiol. 296(4-5), 223–228.
Shalaby, S., Eid, E., Ahmed, O., Abdow, M., 2016. Protective Effect of Ascorbic Acid and Ranitidine on the Indomethacin Induced Gastric Injury in Adult Albino Rats; Light and Electron Microscopy. Int. J. Clin. Dev. Anatomy 2(1), 1–13.
Shao, Li, Lu, A.P., Zhang, L., Li, Y.D., 2003. Anti-*Helicobacter pylori* immunoglobulin G (IgG) and IgA antibody responses and the value of clinical presentations in diagnosis of *Helicobacter pylori* infection in patients with precancerous lesions. World J Gastroenterol. 9(4), 755–758. Published online 2003 Apr 15. doi: 10.3748/wjg.v9.i4.755.
Silva, F., Zaterka, S., Eisig, J., Chehter, E., Chinzon, D., Laudanna, A., 2001. Factors affecting Helicobacter Pylori eradication using a seven-day triple therapy with a proton pump inhibitor, Tinidazole and Clarithromycin, in Brazilian patients with peptic ulcer. Rev. Hosp. Clin. 56(1) São Paulo Jan. /Feb. 2001.On-line version ISSN: 1678-9903.
Tousson, E., 2016. Histo-pathological alterations after a growth promoter boldenone injection in rabbits. Toxicol. Ind. Health 32 (2), 299–305.
Wallace, J.L., 2001. Pathogenesis of NSAID-induced gastroduodenal mucosal injury. Best Pract. Res. Clin. Gastroenterol. 15, 691–703.
Winborn, W., Weser, E., 1983. Scanning electron microscopy of intestinal metaplasia of the human stomach. Gastrointest. Endosc. 29 (3), 201–207.
Wu, K.C., Jacksona, L.M., Galvinb, A.M., Grayc, T., Hawkeya, C.J., Mahidaa, Y.R., 1999. Phenotypic and functional characterisation of myofibroblasts, macrophages, and lymphocytes migrating out of the human gastric lamina propria following the loss of epithelial cells. Gut 44, 323–330.
Xu, L., Surathu, A., Raplee, I., Chockalingam, A., Stewart, S., Walker, L., Sacks, E.L., Patel, V., Zhihua, L., Rouse, R., 2020. The effect of antibiotics on the gut microbiome: a metagenomics analysis of microbial shift and gut antibiotic resistance in antibiotic treated mice. BMC Genom. 21, article number 263.
Yamamoto, I., Fukuda, Y., Mizuta, T., Fukada, M., Nishigami, T., Shimoyama, T., 1995. Serum anti-Helicobacter pylori antibodies and gastritis. Clin. Gastroenterol. 21 (1), S164–S168.
Zaki, S., Mohamed, E., 2014. Effect of glucocorticoids on indomethacin-induced gastric ulcer in the adult male albino rat – histological, morphometric and electron microscopy study. Arch. Med. Sci. 10 (2), 381–388.
Zhang, G.F., Liu, X., Zhang, S., Pan, B., Liu, M.L., 2018. Ciprofloxacin derivatives and their antibacterial activities. Eur. J. Med.

Further Reading

In sperm quality and spermatogenic cells apoptosis following infection in rats. Andrologia 2017; 49: e12621.Pp.1-11. https://doi.org/10.1371/journal.pone.0220636.
Kazemi, M., Eyni, H., Shokri, S., 2017. Co-administration of ginseng and ciprofloxacin ameliorates epididymo-orchitis induced alterations.