International Journal of Pharmacological Research
ISSN: 2277-3312 (Online)
Journal DOI: https://doi.org/10.7439/ijpr **Research Article**

Effect of tamoxifen on ethanol induced gastric ulcer in rats

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Abstract

Background: Tamoxifen is selective estrogen receptor modulators, used in treatment of breast cancer; some literatures reported its impact on the process of peroxidation.
Aim: This study was aimed to evaluate the effect of tamoxifen on ethanol induced gastric ulcer in rats.
Method: Gastric ulcers were induced in Wistar albino rats by oral administration of absolute alcohol (1 ml/200 gm). Antiulcer activity of tamoxifen (0.5 and 10 mg/kg, p.o.) was observed and compared to standard drug (omeprazole 20mg/kg, p.o.), the ulcer index, ulcers numbers, lengths, gastric volume and total gastric acidity were evaluated. Histopathology is performed for confirmation.
Results: tamoxifen in 10mg/kg dose produced a highly significant (P<0.001) decrease in ulcer parameters studied compared to ulcer control. While 0.5mg/kg of tamoxifen produced less significant (P<0.01) effect on gastric volume when compared to ulcer group**.** The anti-ulcer effects of the drug of interest is highly comparable to omeprazole was very, surprisingly; the higher dose of tamoxifen produced even a much significant reduction in gastric volume and ulcers length compared to standard drug.
Conclusion: Tamoxifen shows significant antiulcer activity against ethanol induced gastric ulcers, and this could possibly related to its antioxidant properties.
Keywords: Tamoxifen, Ulcer, Breast cancer, anti-oxidant, cytoprotective.

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***Article History:**
Received: 15/12/2020
Revised: 24/02/2021
Accepted: 28/02/2021
DOI: https://doi.org/10.7439/ijpr.v11i2.5555

QR Code

How to cite: Eljafari M. and Al-Attar A. R. Effect of tamoxifen on ethanol induced gastric ulcer in rats. *International Journal of Pharmacological Research* 2021; 11(02): e5555. Doi: 10.7439/ijpr.v11i2.5555 Available from: https://ssjournals.com/index.php/ijpr/article/view/5555

1. Introduction

Tamoxifen (TAM) is one of the most commonly used exogenous hormonal therapy intended for treatment of breast cancer specifically estrogen receptor (ER)-positive breast cancer [1]. This drug considered as selective estrogen receptor modulator (SERM) which play an agonistic effect on some organs such as the bones and the endometrium but antagonistic effect on estrogen receptors of other organs such as breast tissues [2]. Some Available literatures reported that TAM could interfere with peroxidation process in animal and human systems both *in-vivo* and *in-vitro* [3-5]. This hypothesis leads us to suggest that TAM might improve ethanol induced gastric ulcer, which considered a very common animal model to study ulcer experimentally [6]. Where administration of ethanol found to produce necrotic damage in gastric tissues, subsequent infiltration of inflammatory cells and reduction in secretion of bicarbonate, gastric mucus, and nitric oxide. In addition to reduction in gastric blood flow and induction of oxidative stress [7].

Based on the previously mentioned properties of TAM, it seems that it might have gastroprotective effect on ethanol induced gastric ulcer. Therefore, effect of tamoxifen on ethanol induced gastric ulcer in rats was our aim of the study.

2. Materials and Methods

2.1 Drugs and Chemicals

Tamoxifen citrate (TAM, Ebewe Pharma), stock solution freshly prepared by dissolving it in 0.9% normal saline [8]. Omeprazole (Omeprazole, Actavis) suspended in 1% w/v carboxymethyl cellulose (CMC) and administered orally to the rats in a dosage of 20 mg/kg body weight (5 mL/kg) [9]. All other chemicals and solvents used were of the highest purity grade available.

2.2 Experimental animals

Female Wistar albino rats bred at the Animal house of Department of Pharmacology and Clinical Pharmacy, Tripoli University (Tripoli, Libya) weighing 180-240g were used in the study. They were housed in an ambient temperature of 23°C with a 12h light-dark cycle. Animals were fed a balanced diet and given free access to water. The study was approved by the faculty of pharmacy and the experiments were done according to the ethics guidelines of the Bioethics committee at the Biotechnology research center (BEC-BTRC).

2.3 Treatments and induction of gastric ulcer with Ethanol

The experiment was designed having 5 groups with 6 rats were randomly selected to each group. The rats were fasted for 24 hours with free access to water before ulcer generation. Animals in group one served as a standard ulcerative control group induced by administration of cold absolute alcohol (1 ml/200 g p.o.) per rat weight [10]. The animals in group two (NS) were served as negative control and treated only with normal saline, group three (OMP) which treated with standard drug (20 mg/kg of Omeprazole) administered by gavage. Rats in experimental groups [group four (TAM 0.5) and group five (TAM 10)] were received low and high doses of TAM (0.5 and 10 mg/kg) respectively. The animals were sacrificed 1 hour after ethanol or normal saline administration using an overdose of ether, a midline incision was made with the scalpel. The stomach was excised while the both sides (cardiac and pyloric) were ligated appropriately. Stomach washed by 0.9% normal saline to remove any blood. Each stomach was opened along the greater curvature [11]. Then the effect of the given drugs was analyzed.

2.4 Gross Examination of Gastric Mucosa

After stomach removal, each stomach was pinned flat on cork mat or on paraffin wax-filled Petri dish and examined for ulcers using a hand lens (X10) [12]. Macroscopic examination of the stomach was performed to detect any hemorrhagic lesions on the glandular mucosa. Ulcers of the gastric mucosa appear as inflammation and as bands of hemorrhagic lesions. Using a 0 – 3 scoring system based on the severity of each lesion, as described by Peskar *et al*. [13]. The severity factor was defined according to the length of the lesions: 0 = no lesions; 1 = lesions < 1 mm length; 2 = lesions 2-4 mm length; and 3 = lesions > 4 mm length. The ulcer index (UI) for each rat was calculated as the number of lesions multiplied by their respective severity factor and the mean for each group was taken [13].

2.5 Determination of gastric juice volume and total gastric juice acidity

Before scoring the ulcer, the gastric content volume was measured, then the recovered volume was centrifuged after adding 10 ml of freshly prepared normal saline at 3000 rpm for 10 min. The total gastric acidity was determined by taking one ml of the supernatant and completed to 50 ml with distilled water and titrated against 0.01N NaOH, using1-2 drops of phenolphthalein as indicator [14]. Total gastric acidity was calculated by using the formula:

Acidity (mEq/100g) =

Volume of NAOH* Normality of NAOH * 100/0.1

2.6 Histopathological Evaluation of Gastric Lesion

After determination of the UI, the stomachs of each group were fixed in 10% formalin solution for 24 h. Subsequently, they were dehydrated by immersing them in ascending concentrations of alcohol solutions (70–100%) and in paraffin. Slides of stomach slices of 4–5 µm thickness were prepared and stained with hematoxylin and eosin (H&E) and then analyzed under light microscope at 20× and 40× for pathological changes [15].

2.7 Statistical analysis

Data were presented as mean ± SEM. Statistical significance was determined by one-way analysis of variance (ANOVA) with LSD test as a post hoc. The P values of less than 0.05 were considered to be significant. In statistical analysis, the SPSS version 18.0 program for Windows (SPSS Inc., Chicago, IL, USA) was used.

3. Results

3.1 Gross evaluation of gastric lesions

Intragastric administration of 1ml/200g absolute ethanol developed a consistent and clear pattern of macroscopic damage, as evidenced by the presence of bands of hemorrhagic ulceration, sever gastric ulcers were present in all rats treated with ethanol. The rats given 20mg/kg of omeprazole (standard drug) showed significant improvement ($P<0.001$) in severity, lengths and number of ulcers compared to positive control (ulceration group). similarly results obtained from the TAM groups of either doses (high and low) showed varied degree of improvement in all ulcers macroscopic parameters ($P<0.001$) compared to ethanol treated group (ulceration group). The differences between standard and TAM treated groups did not reach significant levels. Interestingly TAM 10 group showed significant reduction in lengths of ulcers in comparison to standard drug group. Comparing negative control to TAM treated groups; low TAM dose (TAM 0.5) showed significantly higher ulceration numbers ($P<0.01$), lengths ($P<0.01$) and UI ($P=0.001$). While high TAM dose resulted only in a significant higher UI ($p<0.05$) in comparison to negative control, with no significant difference in ulcer length and number. (Figure.1 and table.1).

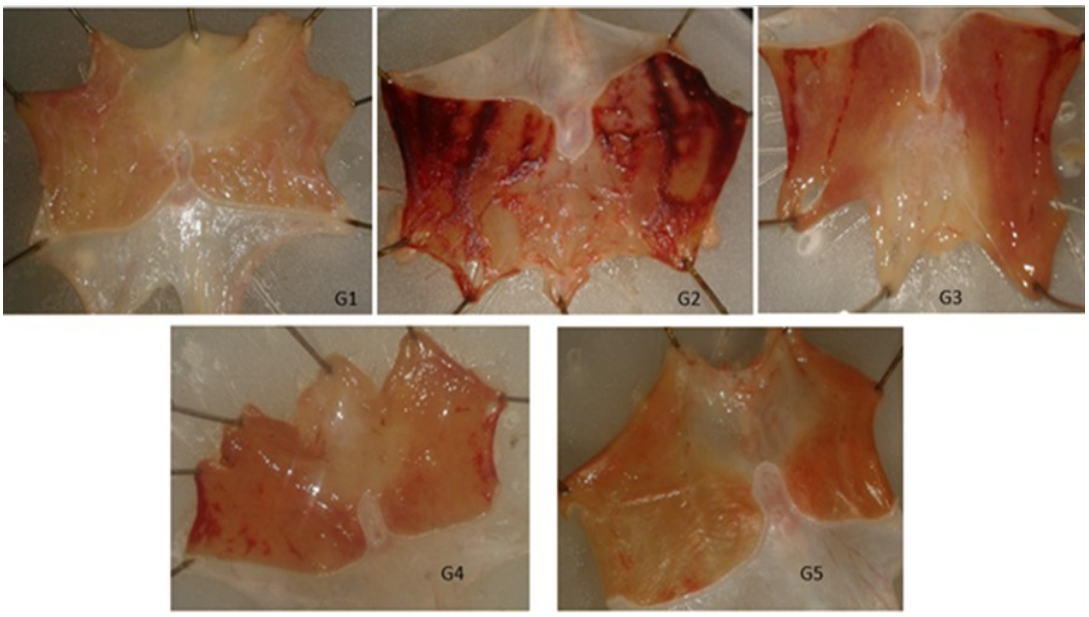

Figure 1: Effect of different doses of TAM on the severity of gastric lesion (gross examination) examined in ethanol-induced gastric ulceration model. **(G1)**NS group: intact gastric mucosa tissues no injuries seen; **(G2)** ethanol ulceration: severe lesions are seen with extensive visible hemorrhagic necrosis of gastric mucosa; **(G3)** OMP group: mild lesions of gastric mucosa are observed compared to the lesions in ethanol (ulcer); **(G4)** low TAM dose (TAM 0.5): few hemorrhagic bands; **(G5)** high TAM dose (TAM 10): nearly normal gastric mucosal tissues with some hyperemia.

Table 1: Effect of TAM on the severity of gastric lesion (ulcer index), length and number of ulcers measured in ethanol-induced gastric ulceration model

Groups	Pre-treatment	UI(mm) X±S.E.M	No. of ulcers	Length of ulcers (mm)
1	NS	-	-	-
2	Absolute ethanol	27.5±1.20	9.2±0.40	109.17±12.2
3	omeprazole	2.83±0.60$^{**\#}$	3.0±0.44$^{**\#\#}$	47.6±10.3$^{**\#\#}$
4	TAM 0.5mg/kg	3.2±0.80$^{**\#\#}$	3.2±0.80$^{**\#\#}$	45.6±16.7$^{**\#\#}$
5	TAM 10 mg/kg	2.0±0.81$^{**\#}$	2.0±0.81$^{**\#}$	15.0±7.2$^{**\wedge}$

Each value is the mean ± S.E.M (n=6). ** (P<0.001) Significant versus ethanol group. #(P<0.05), ## (P<0.01) Significant versus NS group. ^Significant versus OMP group (P≤0.01)

3.2 Effect of Different Treatments on gastric juice volume and total gastric Juice Acidity

Treatment with low dose of TAM before ulcer induction by ethanol produced a significant reduction in gastric volume (**P**<0.01) compared to ulcer group. And more significant reduction (P<0.001) is obtained in group treated with high dose of TAM compared to either OMP group or ulcer group. Similar significant reduction in gastric total acidity (P<0.001) were obtained from OMP group and TAM groups compared to ethanol group (P<0.001). Table.2

Table 2: Effect of TAM on ethanol induced gastric ulcer in rats

Groups	Pre-treatment	Gastric volume (ml)	Gastric total acidity (mEq/100g)
1	NS	0.75±0.11	22.0±1.71
2	Absolute ethanol	4.83±0.03	288.7±19.0
3	omeprazole	3.83±0.10$^{*\#\#}$	26.0±2.70***
4	TAM 0.5mg/kg	3.58±0.30$^{**\#\#\#}$	46.0±7.13***

Each value is the mean ± S.E.M (n=6). *($P \leq 0.05$), ** ($P \leq 0.01$) and *** (P<0.001) Significant versus ethanol group. #(p<0.01) and ##(P<0.01) Significant versus NS group. ^ P<0.001 Significant versus omeprazole group.

3.3 Histological Evaluation of Gastric Lesions

3.3.1 Negative control group:

Examined serial sections from different parts of rat's stomach revealed normal morpho-anatomical structures regarding gastric mucosa, muscularis mucosa, submucosa, muscular coat and serosa. The villi of gastric mucosa were intact with no signs of congestion or hemorrhages, and no exfoliation in the mucosal epithelium was showed. (Figure 2a)

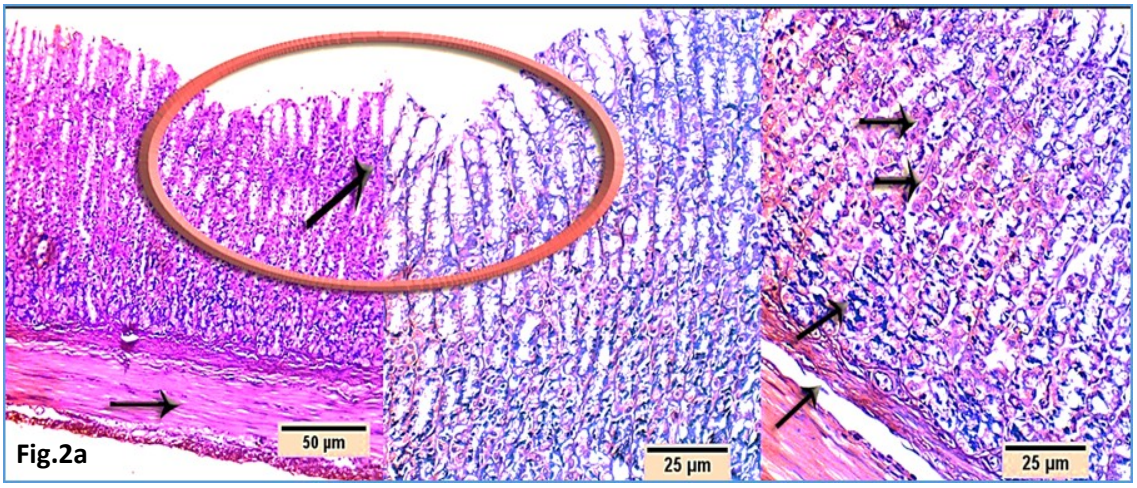

Figure 2a: Photomicrograph from rat's stomach (G 1) showing normal histological structures of all gastric structures (arrows). Scale bars 25 um, 50 um.

3.3.2 Ulcer induced group:

Investigation among ethanol group pointed out erosive and ulcerative changes of different intensities (65-70%) along the mucosa. represented by superficial epithelial degenerative, apoptotic and necrotic changes (DC, NC, APC) with exfoliative and denudated properties. Ulcerative lesions were highly peculiar and manifested as a complete necrotizing change (NZC) in the entire mucosa. (Figure 2b.)

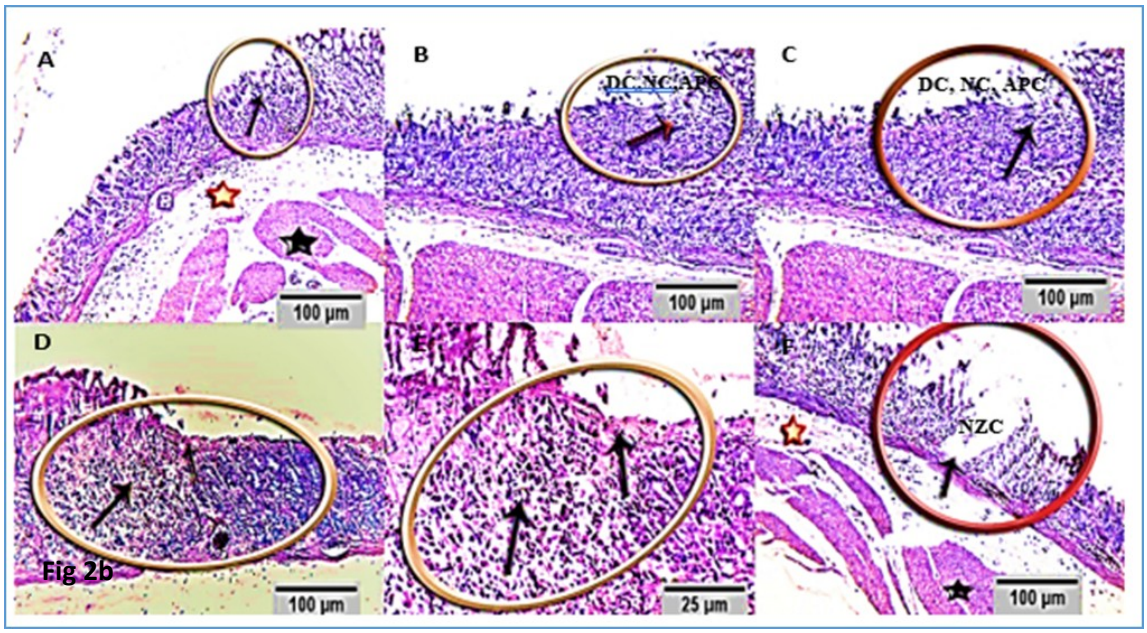

Figure 2b: Photomicrograph from rat's stomach (G2) showing mucosal erosive changes represented by superficial epithelial degenerative, apoptotic and necrotic changes (DC, NC, APC) with exfoliative and denudated properties. Exfoliated cells together with some apoptotic and inflammatory cells are seen in the gastric surface. Ulcerative lesions are manifested as a complete necrotizing change (NZC) in the entire mucosa. The submucosa reveals oedematous changes and inflammatory cells infiltrations (ED&INFC, A, F stars) which extend to the muscular coat evoking focal destructive and disorganization effects (DS& DISO, A, F stars)). Scale bars 25 um, 100um.

3.3.3 Omeprazole treatment group:

This group revealed apparently normal gastric mucosa with healthy mucosal covering epithelium and normal gastric gland Chief, parietal and enterochromaffin cells, however some of the covering mucosal cells and the glandular cells suffered degenerative and apoptotic changes, the submucosa in most of the examined sections showed mild oedematous and inflammatory reaction with predominance of lymphocytes, plasma cells and eosinophils. The blood vessels appeared mildly dilated. The muscular coat and the serosa appeared normal. (Figure 2c)

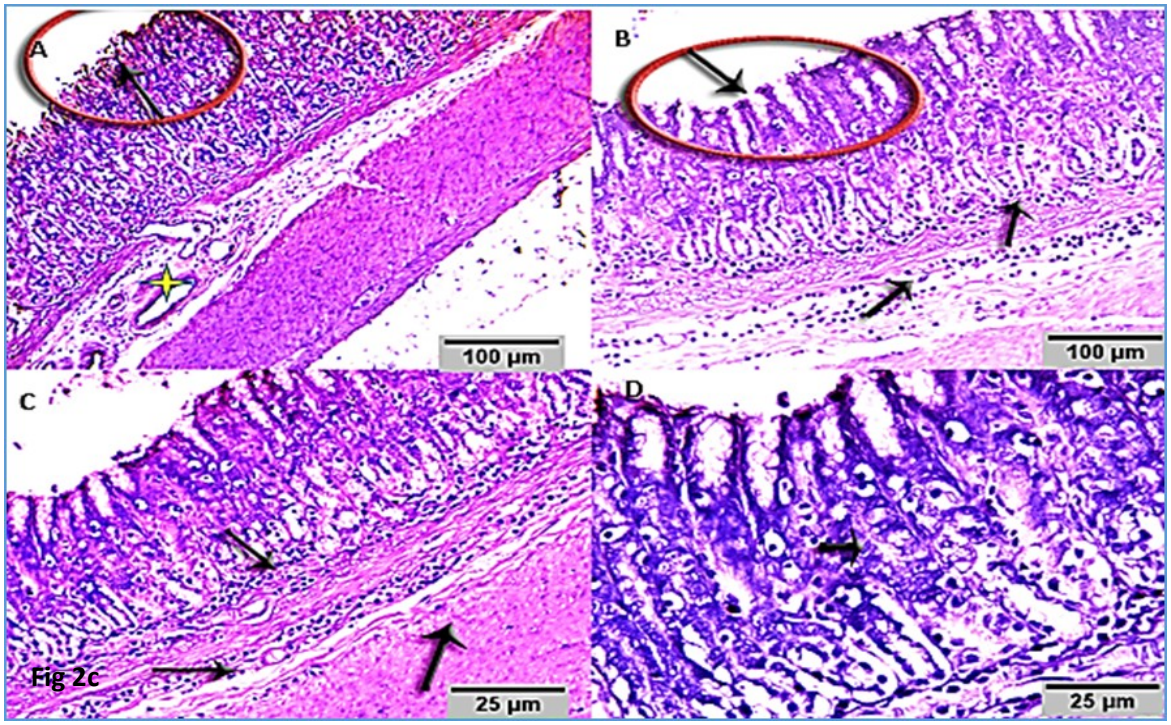

Figure 2c: Photomicrograph from rat's stomach (G 3) showing apparently normal gastric mucosa with healthy mucosal covering epithelium and normal gastric gland Chief, parietal and enterochromaffin cells, however some of the covering mucosal cells and the glandular cells shows degenerative and apoptotic changes (A, B, circles and arrows), the submucosa shows mild oedematous and inflammatory reaction with predominance of lymphocytes, plasma cells and eosinophils (A, B, C, arrows). The blood vessels appear mildly dilated (A, star). Scale bars 25 um, 100um

3.3.4 Tamoxifen treated group (TAM 0.5):

Sections of this group denoted healing process (regenerative changes) in the covering mucosal epithelium with presence of remnant of degenerated and desquamated cells on the top of affected parts. The submucosa revealed mild oedematous reaction, few inflammatory cells and dilated capillaries. (Figure 2d)

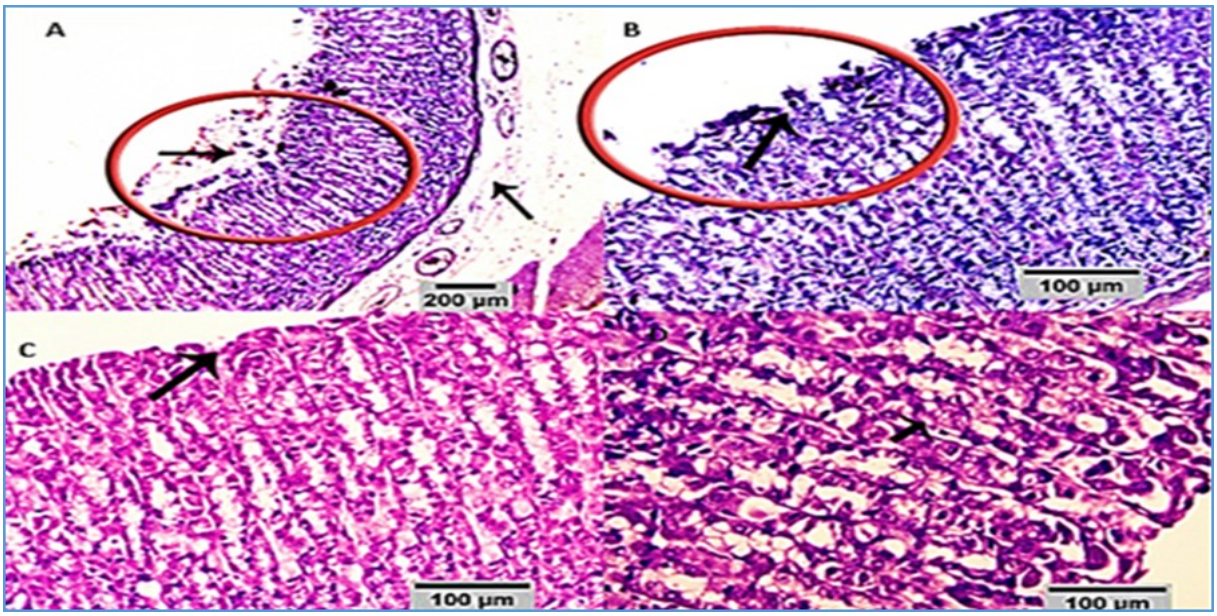

Figure 2d: Photomicrograph from rat's stomach (G 4) showing healing process (regenerative changes) in the covering mucosal epithelium with presence of remnant of degenerated and desquamated cells on the top of affected parts (A, B, circles and arrows). The underlying glandular epithelium appears normal(C, D, arrows). The submucosa shows mild oedematous reaction, few inflammatory cells and dilated capillaries (A, arrow). Scale bars 100um, 200 um.

3.3.5 Tamoxifen -treated group (TAM 10)

All the histo-morphological structures of the gastric mucosa, submucosa, muscle layer and serosa were apparently normal. A few sections showed remnant of regenerating erosive lesions with minimal tissue destruction (3-5%). A few mucosal capillaries were mildly dilated. Neither inflammatory nor oedematous changes were observed. (Figure 2e)

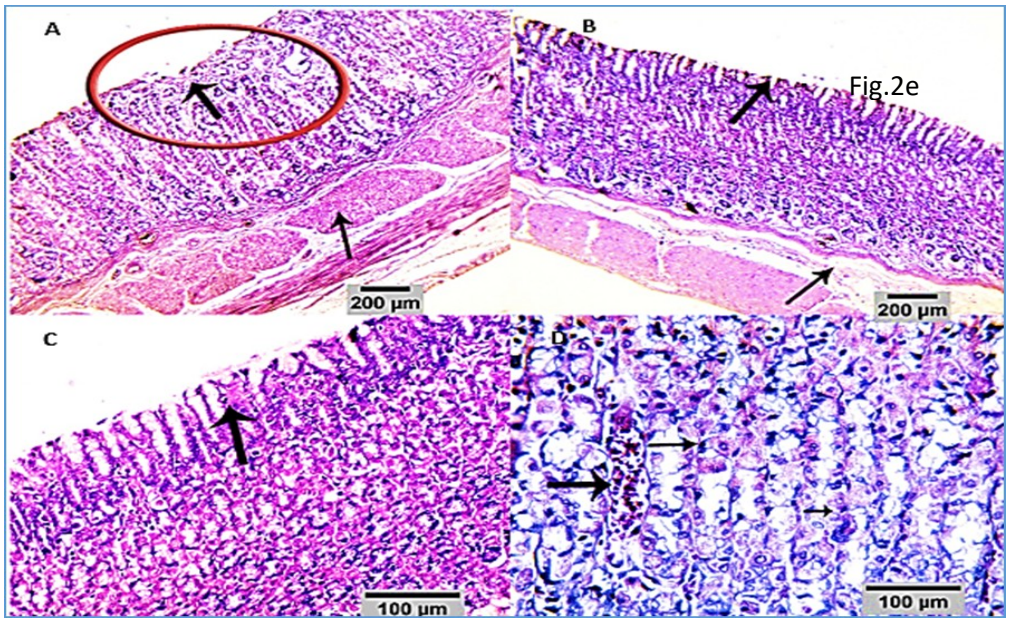

Figure 2e: Photomicrograph from rat's stomach (G 5) showing normal histo-morphological structures of the gastric mucosa, submucosa, muscle layer and serosa. Remnant of regenerating erosive lesions with minimal tissue destruction are seen (A circle and arrow).A few mucosal capillaries appears mildly dilated (D, arrow). Neither inflammatory nor oedematous changes could be seen. Scale bars 100um, 200 um

4. Discussion

This experimental study was designed to investigate the possible protective effect of TAM on ethanol induced gastric lesions in albino rats. Intragastric ethanol administration resulted in massive gastric necrotic damage and subsequent inflammatory cell infiltration. In addition to other subsequent changes like reduction in bicarbonate secretion, gastric mucus, and nitric oxide. Besides that, ethanol reduces the gastric blood flow and induces the oxidative stress by many mechanisms including increasing the production of malondialdehyde and reducing glutathione production [17]. Any protective effect found in the treatment groups on ethanol induced-ulcers in rats might be related to any of the mechanism suggested.

The present study showed significant reduction in number and size of gastric lesions, and also in UI in the groups treated with TAM at both low and high doses compared to ulcer control. One previous study performed on rabbits, has demonstrated that acute treatment with 5mg/kg of oral tamoxifen increases the level of nitric oxide without affecting total antioxidant capacity [8]. This could be responsible in part to the gastroprotective effect of TAM, where nitric oxide help maintaining the integrity of the gastric epithelium and the mucus barrier [18]. Some other in-vivo and in-vitro studies reported that TAM and its metabolite hydroxyl-TAM may exert an inhibitory effect on lipid peroxidation by modifying the structure of the microsomal and liposomal membranes, which decrease the propagation rate of lipid peroxidation [19, 20] and its protective behavior is attributed to free radicals scavenging action [21], where it was found that tamoxifen preserves mitochondrial functions through inhibiting H2O2 formation and GSH depletion in mitochondria of the brain[22].

As stated above, omeprazole and TAM groups showed highly significant ($P<0.001$) attenuation of gastric acidity in comparison to ulcer group. And this highly significant reduction in total gastric acidity observed in this study strongly suggests that TAM may have an inhibitory effect on gastric acid secretion. Where the gastric fluid volume in TAM treated groups was suppressed in a dose dependent manner, and 10mg/kg of TAM caused highly significant reduction in gastric acid secretion compared to ulcer control, and that effect exceeded the effect produced by the standard drug (20mg/kg of omeprazole). Which is routinely used in gastric ulcer because of its ability in suppressing gastric acid secretion by inhibiting the proton pumps [23]. The hypothesis of TAM gastric acid secretion suppressive behavior is supported by Huh and his group (2012), where they found that treatment of normal mice with a single ≥3 mg/20 g body weight dose of TAM leads to

apoptosis of >90% of all gastric parietal cells (PCs) and metaplasia of zymogenic chief cells within 3 days of treatment. Though the TAM doses used in the current study is much lower than the dose used by Huh group [24].

In conclusion, this study suggested the possible gastroprotective effect of TAM against gastric ulcer induced by ethanol, which found to be dose dependent. This regenerative effect of TAM is confirmed by histopathology, but further investigations are required to confirm the exact mechanism underlining the gastroprotective effect of TAM.

Acknowledgment

The authors are grateful to Dr. Taha Ben Yaseid the head of Pathology Department in Tripoli Medical Center (TMC) for providing facilities for photographing the slides of histopathology.

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