Effect of Tamoxifen on Ethanol Induced Gastric Ulcer in Rats

Article · April 2021

DOI: 10.46610/RTPScR.2021.v03i01.005

2 authors:

Malak Jafari
University of Tripoli
2 PUBLICATIONS **0** CITATIONS
SEE PROFILE

Al-Asayed R. Al-Attar
Faculty of Veterinary Medicine,Zagazig University,Egypt
63 PUBLICATIONS **84** CITATIONS
SEE PROFILE

Recent Trends in Pharmaceutical Sciences and Research

Volume-3, Issue-1 (January-June, 2021)

www.matjournals.com

https://doi.org/10.46610/RTPScR.2021.v03i01.005

Effect of Tamoxifen on Ethanol Induced Gastric Ulcer in Rats

Malak M. Eljafari[1*], Al-Asayed R. Al-Attar[2]
Assistant Lecturer
[1]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, University of Tripoli, Tripoli, Libya
[2]Department of Pathology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt

*Corresponding Author: m.jafari@uot.edu.ly

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: The aim of this study was 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 g). 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 0.5 mg/kg of tamoxifen produced a less significant (P<0.01) effect on gastric volume when compared to the ulcer group. The anti-ulcer effects of the drug of interest is highly compared to omeprazole was very, surprisingly; the higher dose of tamoxifen produced even a much significant reduction in gastric volume and ulcers length compered to standard drug. Conclusion: Tamoxifen shows significant antiulcer activity against ethanol induced gastric ulcers, and this could possibly related to its antioxidant properties.

Keywords-- Anti-oxidant, Breast cancer, cytoprotective, Tamoxifen, Ulcer

INTRODUCTION

Exogenous hormonal therapy includes several drugs like Tamoxifen (TAM), which is one of the most commonly used treatments intended for management of breast cancer specifically estrogen receptor (ER)-positive breast cancer [1]. This drug plays a dual effect, where it has an agonistic effect on some organs such as the bones and the endometrium but an antagonistic effect on estrogen receptors of other organs such as breast tissues and this effect known as selective estrogen receptor modulator (SERM) [2]. Some available literature 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]. Ethanol administration were found to produce necrotic damage in gastric tissues, subsequent infiltration of inflammatory cells and reduction in many secretory functions of many substances like bicarbonate, gastric mucus and nitric oxide. Besides that the gastric blood flow reduction and oxidative stress induction were also noticed [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.

MATERIALS AND METHODS

Drugs and Chemicals

Tamoxifen citrate (TAM, Ebewe Pharma), stock solution freshly prepared by dissolve it in 0.9% normal saline [8].

https://doi.org/10.46610/RTPScR.2021.v03i01.005

Omeprazole (Omeprazole, Actavis) was administered by gavage in suspension form using 1% w/v carboxymethyl cellulose (CMC), and given to 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.

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°±2C with a 12h light-dark cycle. Animals were fed a standard diet with free access to water. The study was approved by the Faculty of Pharmacy/University of Tripoli and the experiments were done according to the ethics guidelines of the Bioethics committee at the Biotechnology Research Center (BEC-BTRC), and registered under No. (BEC-BTRC 36-2020).

Treatments and Induction of Gastric Ulcer with Ethanol

The experiment was designed by having 5 groups with randomly selected rats and each group has 6 animals. All animals were fasted for 24 before ulcer generation with free access to water. 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.

Grossly Examination of Gastric Mucosa

After removal of the stomach, each stomach was pinned flat on either a cork mat or on paraffin wax-filled Petri dish and examined for ulcers using a hand magnifying lens (X10) [12]. Gross examination of the stomach was performed to detect any hemorrhagic lesions on the glandular mucosa. Gastric mucosal ulcers appear as inflammation and as bands of hemorrhagic lesions. The scoring system used in this study was (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 where 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].

Determination of Gastric Juice Volume and Total Gastric Juice Acidity

Before scoring the ulcer, the gastric content volume was measured and 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, using 1-2 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

Microscopic Evaluation of Gastric Lesion (Histopathological Examination)

After determination of the ulcer index (UI), the stomachs dissected from each group were carefully fixed in 10% formalin solution for 24 h. Subsequently, the dehydration process was performed by immersing them in ascending concentrations of alcohol solutions (70–100%) then embedded in paraffin. The stomach sliced into slides of 4–5 µm thickness

https://doi.org/10.46610/RTPScR.2021.v03i01.005

then prepared and stained with hematoxylin and eosin (H&E) to be analyzed under light microscope at 20x and 40x for pathological changes [15].

Statistical Analysis

The obtained data were presented as mean ± SEM. The one-way analysis of variance (ANOVA) with LSD test as a post-hoc was used to determine the statistical significance. 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.

RESULTS

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, the high TAM dose result only in a significantly higher UI ($p<0.05$) in comparison to the negative control, with no significant difference in ulcer length and number (Fig.1 & Table 1).

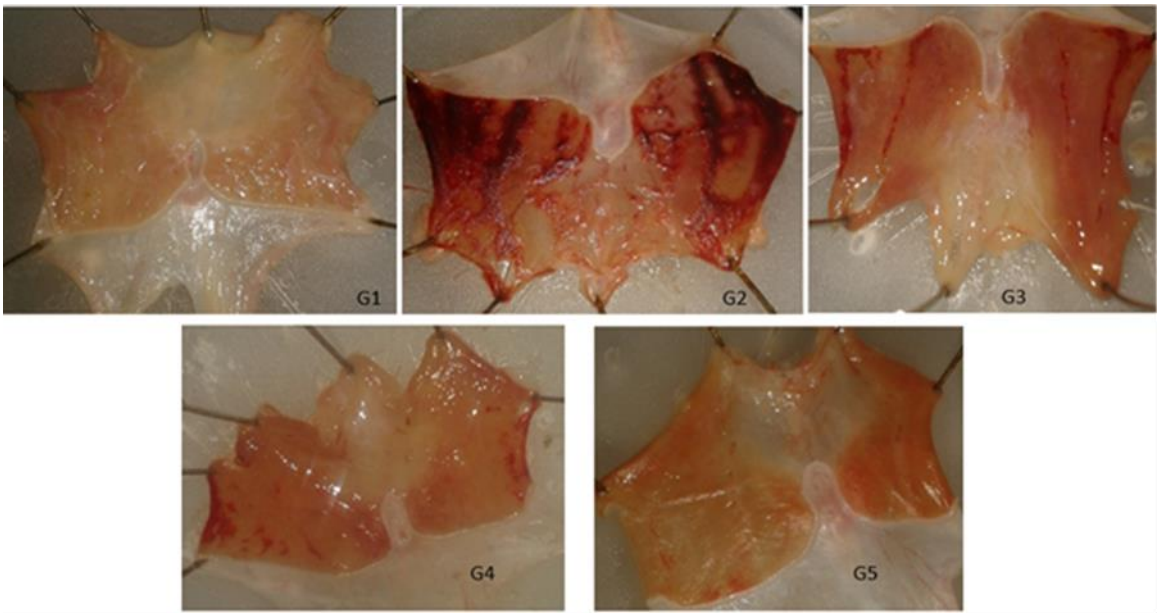

Figure 1*: Effect of different doses of TAM on the severity of gastric lesion (gross examination) examined in ethanol-induced gastric ulceration model.*

(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;

www.matjournals.com

https://doi.org/10.46610/RTPScR.2021.v03i01.005

(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)

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.

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

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) as showing below in 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.

Histological Evaluation of Gastric Lesions

Negative Control Group

Examined serial sections from different parts of rats stomach revealed normal morpho-anatomical structures regarding the 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 as observed in below fig. 2(a).

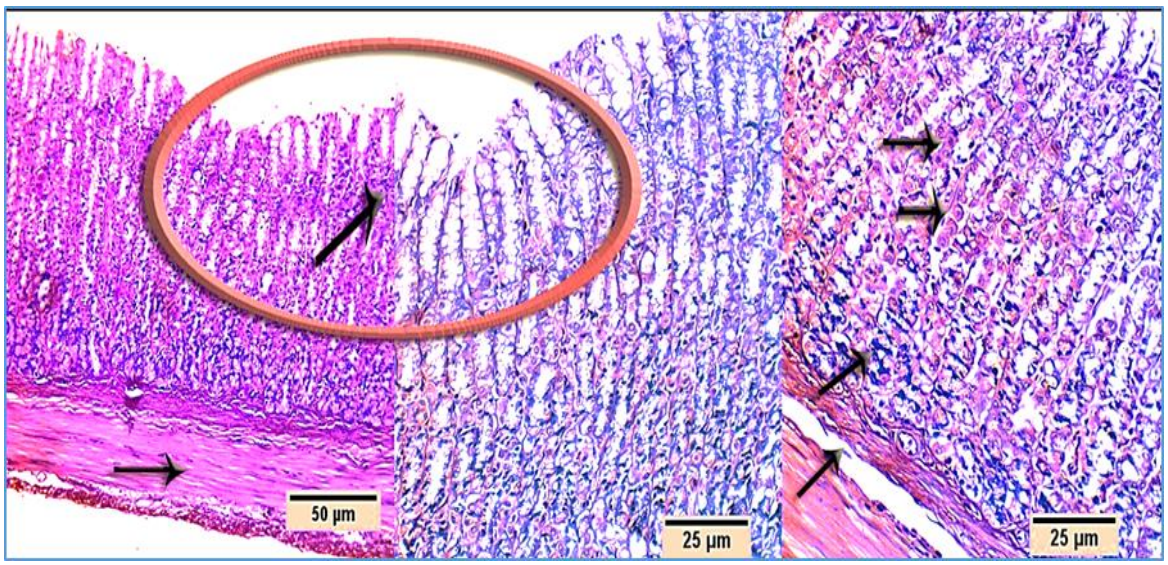

***Figure 2 (a)**: Photomicrograph from rat's stomach (G 1) showing normal histological structures of all gastric structures (arrows). Scale bars 25 um, 50 um.*

Ulcer Induced Group

The 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 shown in Fig. 2(b).

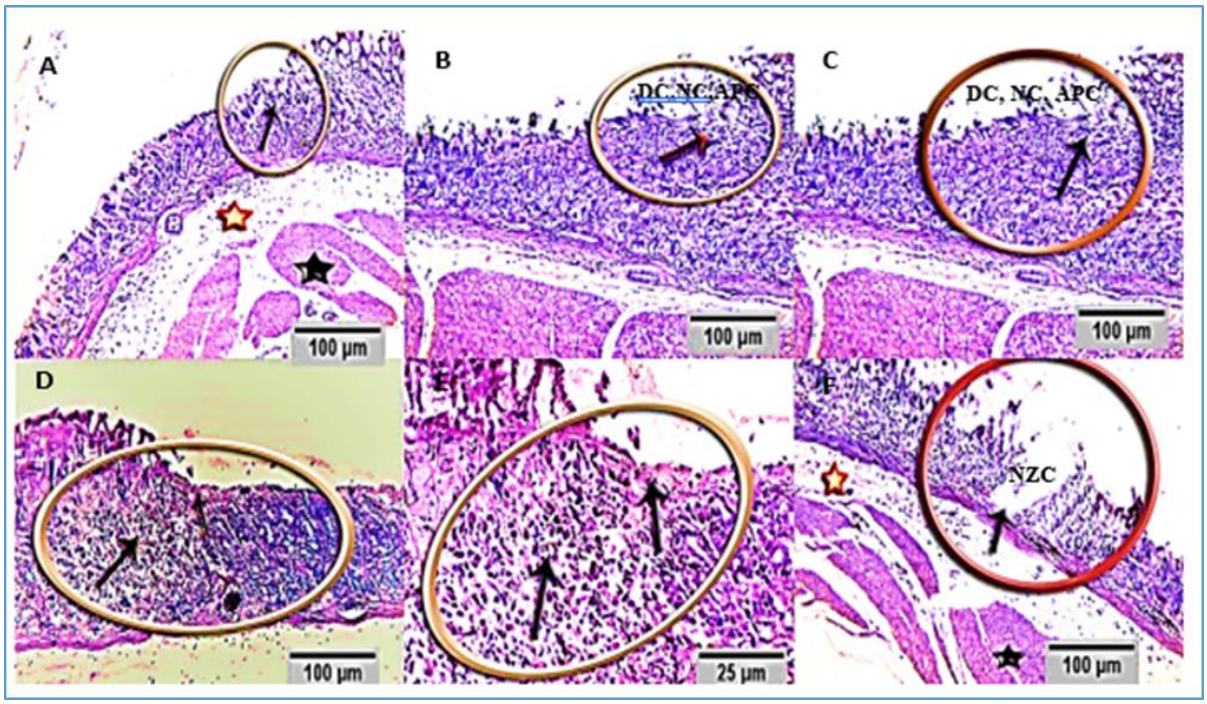

***Figure 2(b):** Photomicrograph from rat's stomach (G2) showing mucosal erosive changes represented by superficial epithelial degenerative, apoptotic and necrotic changes (DC, NC, APC) with exfoliative and denudated properties. Exfoliated cells together with some apoptotic and inflammatory cells are 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.

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 as shown in Fig. 2(c).

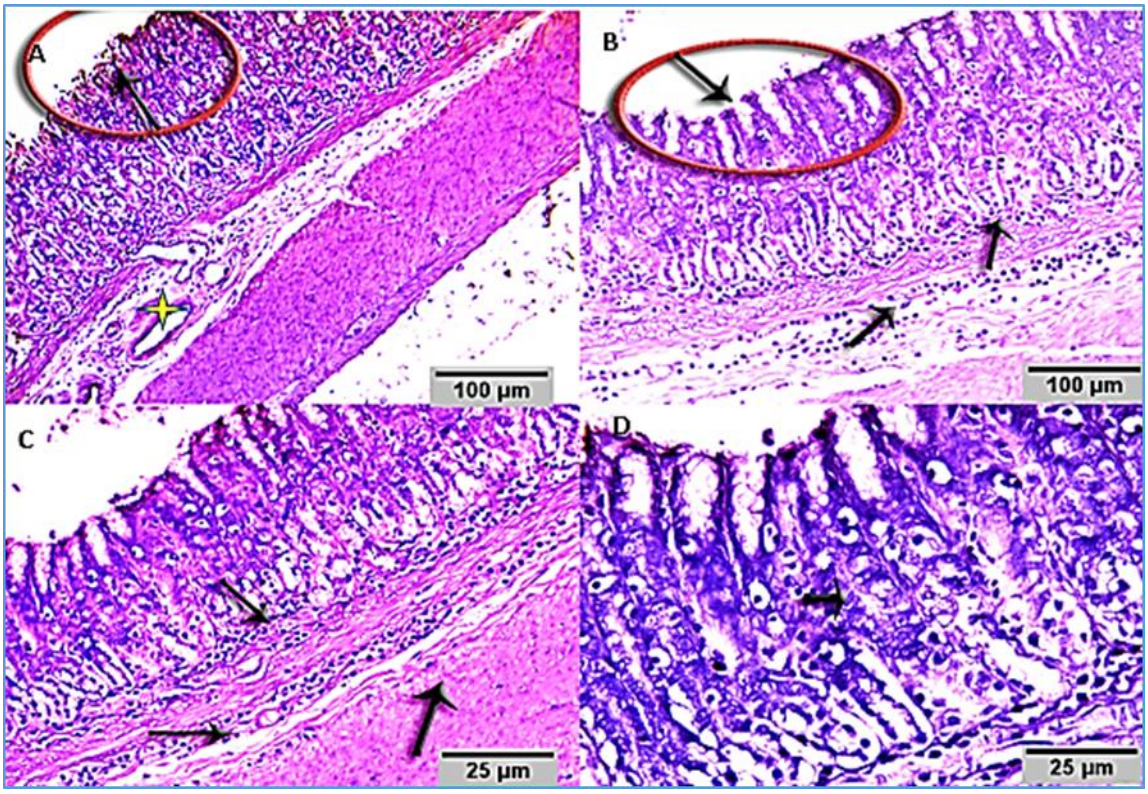

***Figure 2(c)**: Photomicrograph from rat's stomach (G3) showing apparently normal gastric mucosa with healthy mucosal covering epithelium and normal gastric gland Chief , parietal and enterochromaffin cells, however some of the covering mucosal cells and the glandular cells shows degenerative and apoptotic 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.*

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 as shown in Fig. 2(d).

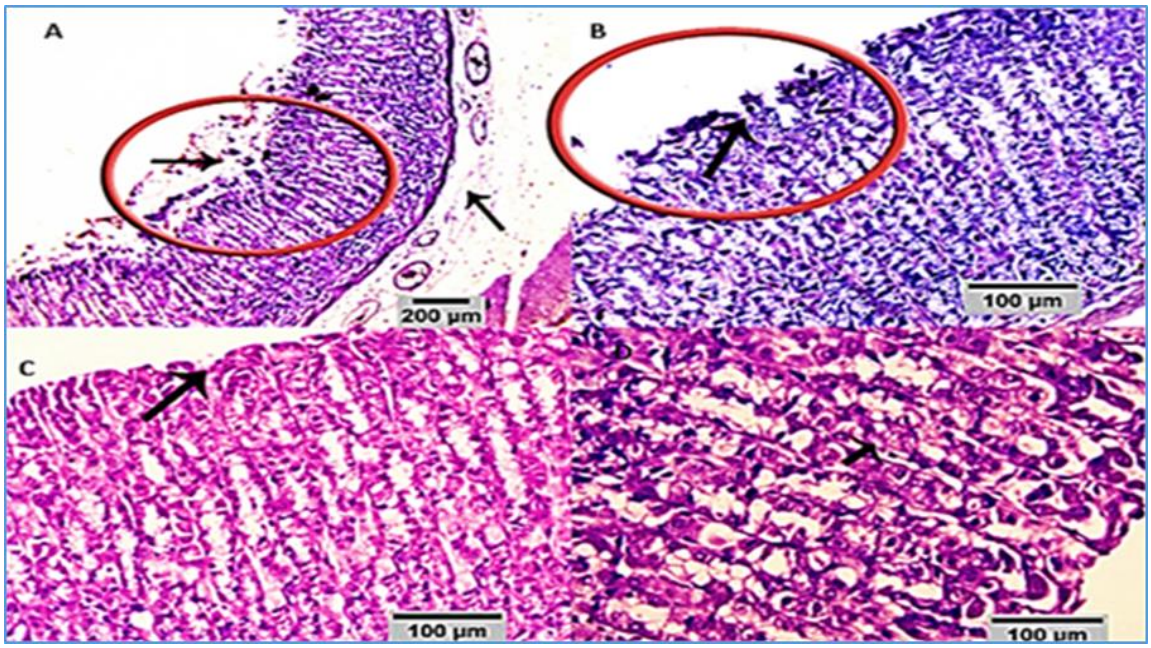

Figure 2(d): *Photomicrograph from rats stomach (G4) showing healing process (regenerative changes) in the covering mucosal epithelium with presence of remnant of degenerated and desquamated cells on the top of affected parts (A, B, circles and arrows). The underlying glandular epithelium appears normal(C, D, arrows). The submucosa shows mild oedematous reaction, few inflammatory cells and dilated capillaries (A, arrow). Scale bars 100um, 200 um.*

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 as shown in Fig. 2(e).

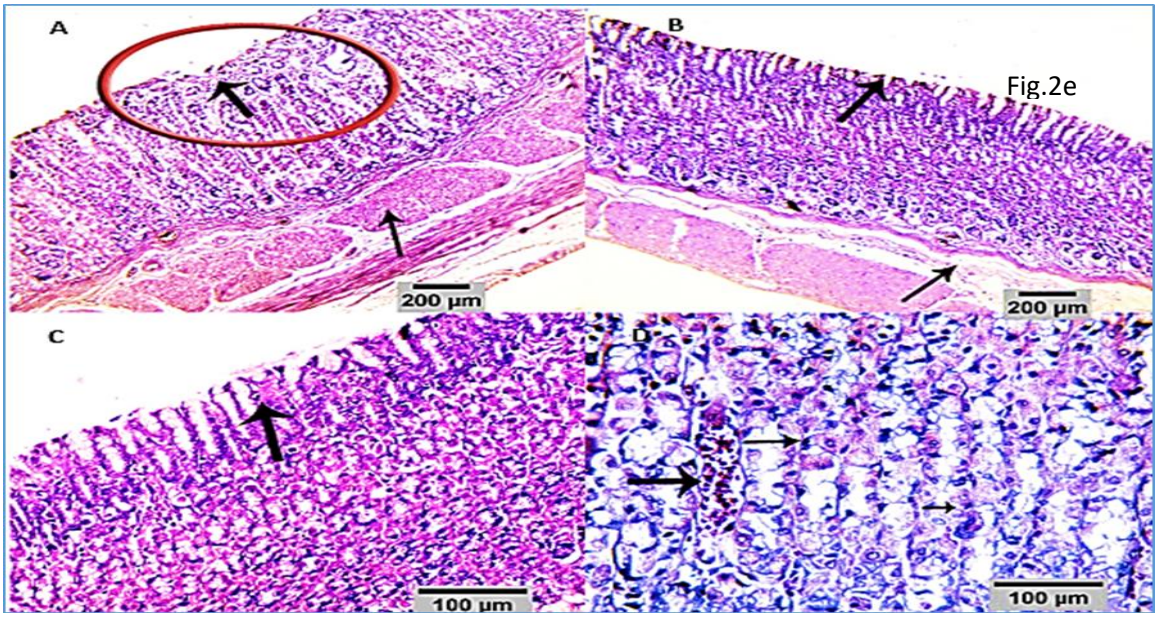

Figure 2(e): *Photomicrograph from rat's stomach (G 5) showing normal histo-morphological structures of the gastric mucosa, submucosa, muscle layer and serosa. Remnant of regenerating erosive lesions with minimal tissue destruction are seen (A circle and arrow). A few mucosal capillaries appears mildly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

https://doi.org/10.46610/RTPScR.2021.v03i01.005

DISCUSSION

This experimental study was designed to investigate the possible protective effect of TAM on ethanol induced gastric lesions in albino rats. Intragastric absolute ethanol administration resulted in massive gastric necrotizing damage and subsequent infiltration of inflammatory cells. In addition to other subsequent changes including reduction in gastric mucus, bicarbonate secretion, 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 effect could be responsible in part for 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 H_2O_2 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 extensively used in models of experimental gastric ulcer because of its ability to suppress 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 dose of ≥3 mg/20 g body weight dose of TAM caused an apoptotic effect of >90% of all gastric parietal cells (PCs) and metaplasia of zymogenic chief cells within short period of time (3 days of treatment) Though the TAM doses used in the current study is much lower than the dose used by Huh group [24].

CONCLUSION

In conclusion, this study suggested the possible gastro protective 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 gastro protective 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.

REFERENCES

1. Chang M. (2012). Tamoxifen resistance in breast cancer. *Bimolecular Theraputics (Seoul)*. 20(3), 256-67. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3794521/
2. Martinkovich, S., Shah, D., Planey, S. L., & Arnott, J. A. (2014). Selective estrogen receptor modulators: tissue specificity and clinical utility. *Clinical interventions in aging*, *9*, 1437. DOI: https://doi.org/10.2147/cia.s66690
3. Thangaraju, M., Vijayalakshimi, T., & Sachdanandam, P. (1994). Effect of

tamoxifen on lipid peroxide and antioxidative system in postmenopausal women with breast cancer. *Cancer*, *74*(1), 78-82. DOI: https://doi.org/10.1002/1097-0142(19940701)74:1%3C78::AID-CNCR2820740114%3E3.0.CO;2-L

4. Hernández-Esquivel, L., Zazueta, C., Buelna-Chontal, M., Hernández-Reséndiz, S., Pavón, N., & Chávez, E. (2011). Protective behavior of tamoxifen against Hg2+-induced toxicity on kidney mitochondria: In vitro and in vivo experiments. *The Journal of steroid biochemistry and molecular biology*, *127*(3-5), 345-350. DOI: https://doi.org/10.1016/j.jsbmb.2011.07.004
5. Belenichev, I. F., Odnokoz, O. V., Pavlov, S. V., Belenicheva, O. I., & Polyakova, E. N. (2012). The neuroprotective activity of tamoxifen and tibolone during glutathione depletion in vitro. *Neurochemical Journal*, *6*(3), 202-212. DOI: https://doi.org/10.1134/S181971241203004X
6. Patel, R., Baker, S. S., Liu, W., Desai, S., Alkhouri, R., Kozielski, R., & Zhu, L. (2012). Effect of dietary advanced glycation end products on mouse liver. *PLoS One*, *7*(4), e35143. DOI: https://doi.org/10.1371/journal.pone.0035143
7. Sistani Karampour, N., Arzi, A., Rezaie, A., Pashmforoosh, M., & Kordi, F. (2019). Gastroprotective effect of zingerone on ethanol-induced gastric ulcers in rats. *Medicina*, *55*(3), 64. DOI: https://doi.org/10.3390/medicina55030064
8. Atakisi, E., Kart, A., Atakisi, O., & Topcu, B. (2009). Acute tamoxifen treatment increases nitric oxide level but not total antioxidant capacity and adenosine deaminase activity in the plasma of rabbits. *Eur Rev Med Pharmacol Sci*, *13*(4), 239-243. Available at: https://pubmed.ncbi.nlm.nih.gov/19694340/
9. Tayeby, F., Salman, A. A. A., Kamran, S., Khaing, S. L., & Salehen, N. A. B. (2017). Ulcer prevention effect of 3, 4, 5-tihydroxy-N0-[(2-methyl-1H-indol-3yl) methylidene] benzohydrazide in HCl/ethanol-induced gastric mucosal damage in rats. *International journal of medical sciences*, *14*(13), 1317. DOI: https://dx.doi.org/10.7150%2Fijms.20984
10. Süleyman, H., Akçay, F., & Altinkaynak, K. (2002). The effect of nimesulide on the indomethacin-and ethanol-induced gastric ulcer in rats. *Pharmacological research*, *45*(2), 155-158. DOI: https://doi.org/10.1006/phrs.2001.0933
11. Gregory, M., Divya, B., Mary, R. A., Viji, M. H., Kalaichelvan, V. K., & Palanivel, V. (2013). Anti–ulcer activity of Ficus religiosa leaf ethanolic extract. *Asian Pacific journal of tropical biomedicine*, *3*(7), 554-556. DOI: https://doi.org/10.1016/S2221-1691(13)60112-4
12. Ezgallaey, H. M., Abuskhuna, S. M., & Dugani, A. M. (2016). Evaluation of Gastroprotective Activity of 1-Hydroxy-2-phenylbenzimidazole in Ethanol-induced Gastric Ulcers in Rats. *Libyan International Medical University Journal*, *1*(2), 82-92. DOI: https://dx.doi.org/10.21502/limuj.009.01.2016
13. Peskar, B. M., Ehrlich, K., & Peskar, B. A. (2002). Role of ATP-sensitive potassium channels in prostaglandin-mediated gastroprotection in the rat. *Journal of Pharmacology and Experimental Therapeutics*, *301*(3), 969-974. Available at: https://jpet.aspetjournals.org/content/301/3/969.short
14. Inas, Z. A., Hala, A. K., & Gehan, H. H. (2011). Gastroprotective effect of Cordia myxa L. fruit extract against indomethacin-induced gastric ulceration in rats. *Life Sci J*, *8*(3), 433-445. Available at: https://www.researchgate.net/profile/Gehan-Heeba/publication/233910423_Gastroprotective_Effect_of_Cordia_Myxa_L_Fruit_Extract_against_Indomethacin-Induced_Gastric_Ulceration_in_Rats/links/555364d208ae6fd2d81f1bd3/Gastroprotective-Effect-of-Cordia-Myxa-L-Fruit-Extract-against-Indomethacin-Induced-Gastric-Ulceration-in-Rats.pdf

15. Anoop, A., & Jegadeesan, M. (2003). Biochemical studies on the anti-ulcerogenic potential of Hemidesmus indicus R. Br. var. indicus. *Journal of ethnopharmacology*, *84*(2-3), 149-156. DOI: https://doi.org/10.1016/S0378-8741(02)00291-X
16. Abdulla, M. A., Ahmed, K. A. A., Al-Bayaty, F. H., & Masood, Y. (2010). Gastroprotective effect of Phyllanthus niruri leaf extract against ethanol-induced gastric mucosal injury in rats. *African Journal of Pharmacy and Pharmacology*, *4*(5), 226-230. DOI: https://doi.org/10.1016/j.cbi.2015.01.015
17. El-Maraghy, S. A., Rizk, S. M., & Shahin, N. N. (2015). Gastroprotective effect of crocin in ethanol-induced gastric injury in rats. *Chemico-biological interactions*, *229*, 26-35. Available at: https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.717.6201&rep=rep1&type=pdf
18. Lanas, A. (2008). Role of nitric oxide in the gastrointestinal tract. *Arthritis research & therapy*, *10*(2), 1-6. DOI: https://doi.org/10.1186/ar2465
19. Wiseman, H., Laughton, M. J., Arnstein, H. R., Cannon, M., & Halliwell, B. (1990). The antioxidant action of tamoxifen and its metabolites Inhibition of lipid peroxidation. *FEBS letters*, *263*(2), 192-194. DOI: https://doi.org/10.1016/0014-5793(90)81371-T
20. Custódio, J. A., Dinis, T. C., Almeida, L. M., & Madeira, V. M. (1994). Tamoxifen and hydroxytamoxifen as intramembraneous inhibitors of lipid peroxidation. Evidence for peroxyl radical scavenging activity. *Biochemical pharmacology*, *47*(11), 1989-1998. DOI: https://doi.org/10.1016/0006-2952(94)90073-6
21. Moreira, P. I., Custódio, J. B., Oliveira, C. R., & Santos, M. S. (2005). Brain mitochondrial injury induced by oxidative stress-related events is prevented by tamoxifen. *Neuropharmacology*, *48*(3), 435-447. DOI: https://doi.org/10.1016/j.neuropharm.2004.10.012
22. Ek, R. O., Yildiz, Y., Cecen, S., Yenisey, C., & Kavak, T. (2008). Effects of tamoxifen on myocardial ischemia-reperfusion injury model in ovariectomized rats. *Molecular and cellular biochemistry*, *308*(1), 227-235. DOI: https://doi.org/10.1007/s11010-007-9633-0
23. Shin, J. M., & Sachs, G. (2008). Pharmacology of proton pump inhibitors. *Current gastroenterology reports*, *10*(6), 528-534. DOI: https://doi.org/10.1007/s11894-008-0098-4
24. Huh, W. J., Khurana, S. S., Geahlen, J. H., Kohli, K., Waller, R. A., & Mills, J. C. (2012). Tamoxifen induces rapid, reversible atrophy, and metaplasia in mouse stomach. *Gastroenterology*, *142*(1), 21-24. DOI: https://doi.org/10.1053/j.gastro.2011.09.050