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Anti-Ulcer Activity of Pistacia Atlantica and Punica Granatum Hydroalcoholic Extract in Comparison with Omeprazole in Male Wister Rats

Asseel Abdulateef Abdulzahra
Department of Pathology and Poultry Disease, Faculty of Veterinary, University of Kufa, Najaf, Iraq

Hutheyfa Abdulhussein Ali Al-Salih
Department of Pathology and Poultry Disease, Faculty of Veterinary, University of Kufa, Najaf, Iraq

***Abstract*---**The current study was conducted to investigate the protective effects of *Pistacia Atlantica* (mastic gum) hydro-alcoholic extract and *Punica Granatum* (pomegranate) peel hydro-alcoholic extract treatment against alcohol-induced peptic ulcer in Wister rat and to compare this protective effects between each other and with omeprazole as the most common drug in treatment of peptic ulcer. Thirty five male Wister rats were assigned into five groups (seven rats each group). C- Group rats were received normal saline for one week. C+ group rats were received normal saline for one week before receiving one dose of absolute ethanol. OME group rats were received normal saline for one week and one dose of omeprazole one hour before receiving one dose of absolute ethanol. MG group rats were received *Pistacia Atlantica* hydro-alcoholic extract for one week before receiving one dose of absolute ethanol. PG group rats were received *Punica Granatum* hydro-alcoholic extract daily for one week before receiving one dose of absolute ethanol. The gross and histopathology lesion of gastric ulcer were assessed. Ulcer index results showed a significant ($p<0.05$) differences between C+ group and other groups, However, the ulcer index of PG group was significant ($p<0.05$) compared with OME group.

***Keywords*---**omeprazole, peptic ulcer, pistacia atlantica, punica granatum, rabbits.

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***Corresponding author**: Abdulzahra, A. A.; Email: asseela.abdulzahra@uokufa.edu.iq*
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Introduction

Gastric ulcer (GU) is a common gastrointestinal condition that affects millions of people around the world. According to the survey, Gastric ulcer affects 20–60 persons out of every 100,000 people, accounting for 5–10 percent of global mortality (Sung et al., 2010). It is a break in the normal gastric mucosa integrity that extends through the muscularis mucosa into the sub-mucosa or deeper (Brown & Wilson, 1999). Several medicinal herbs are used in the traditional medicine with anti-peptic ulcer properties. The most well-known species of Pistacia is mastic gum (Pistacia Atlantica), which is found throughout the Mediterranean and Middle East (Mahjoub et al., 2018). It can help with both upper and lower gastrointestinal problems. The mastic gum is a stomach tonic and it is used for dyspepsia, stomach ulcers, esophagitis and gastritis. Also, mastic gum is effective in nausea, vomiting, colic, hemorrhoid, anal fissures and intestinal worms (Mahmood et al., 2011).

Pomegranate (Punica Granatum L.) is a famous table fruit in tropical and subtropical regions of the world. It belongs to the Punicaceae family and the Punica L. Genus, It is used as an anti-parasitic agent, a “blood tonic” in addition to heal aphthae, diarrhea, and ulcers. Moreover, Pomegranate serves as a remedy for diabetes (Moghaddam et al., 2014). The current study was conducted to investigate the protective effect of Pistacia Atlantica and Punica Granatum L.on ulcer.

Material and Method

A-plant preparation
Pistacia atlantica l hydro-alcoholic extract

Pistacia Atlantica was obtained from local markets and ground to powder form using fine grinder. 300 MG. of Pistacia Atlantica powder was soaked in 1 liter of 70% ethanol for 72 hours, then the solution was filtered and extracted using a rotary evaporator. Rats have received the Pistacia Atlantica extract at the dose of 400 mg/kg of body weight daily by gavages for one -week pre-ethanol administration (Sistani Karampour et al., 2019).

Punica granatum hydro-alcoholic extract

Pomegranate peel was dried and ground to powder form using fine grinder. 50 MG. of Pomegranate peel powder was soaked 500 ml in 80% methanol for 72 hours, then the solution was filtered and extracted using a rotary evaporator. Rats were received the pomegranate peel extract at dose of 100 mg/kg of body weight daily by gavages for one week pre-ethanol administration (Moghaddam et al., 2014).

Chemicals

Omeprazole was used at the dose of 20 mg/kg of body weight 1 hour pre-ethanol administration (Choice et al., 2019).

Experimental design

In this experimental, thirty five male Wister rats with weight range 150-200 g. at eight weeks-old are used, they were placed in plastic cages (two rats per cage) with wood chips for bedding, fed with commercial chew and tap water for drinking (ad libitum). The Rats were assigned randomly and acclimatized for one week pre-experimental period. The rats were divided into five groups, namely C-, C+, OME, MG and PG. C- group rats were received normal saline by gavage daily for one week. C+ group rats were received absolute ethanol at the dose of 5 ml/kg of body weight daily for one week . OME group rats were received omeprazole at the dose of 20 mg/kg of body weight daily for one week. MG group rats were received Pistacia Atlantica hydro-alcoholic extract at the dose of 400 mg/kg of body weight daily for one week. PG group rats were received Punica Granatum hydro-alcoholic extract at the dose of 100 mg/kg of body weight daily for one week.

Parameters measured

Gross Pathology (Ulcer Index)

Scoring of ulcer/ratings was made as described by Takagi and Okabe (TAKAGI & OKABE, 1968) : (0) no lesion, (1) one – two mucosal petechial, (2) one to five small lesions (1-2 mm), (3) more than five small lesions or one intermediate lesion (3-4 mm), (4) two to more intermediate lesions or one gross lesion (>4 mm), (5) perforated ulcers.
The ulcer index and the percentage protective ratio are, respectively, given by the following equation:

- ulcer index (UI) = (total ulcer score)/(number of animal ulcerated) .
- percentage protective ratio =(UI of ulcerogen treatedgroup)/(UI of ulcerogen treated)-(UI of tretment group)/(UI of ulcerogen treted)

Histopathology

Stomach sample were collected immediately after necropsy and fixed in 10% formalin for 48 hours. Tissue samples were sliced to 0.5 cm thick and placed in plastic cassettes for dehydration and clearing using an automated tissue processor, before embedded in paraffin. The tissue samples were then trimmed and sectioned at 4 μm thickness. Then the tissue sections were placed carefully in water bath and mounted on glass slides using a hot plate. Stomach tissue sections were deparaffinized by two changes of xylene for 2 minutes each and rehydrated by three changes of different ethanol dilution (100%, 90% and 70%) for 2 minutes each, respectively, and stained with hematoxylin–eosin (H&E) stain. Tissue sections were observed and examined using a light microscope at 40x, 100x, 200x and 400x magnifications.

Statistical analysis

Ulcer index results were conducted by using ONE WAY ANOVA test, Kruskal Wallis non-parametric ANOVA test and Mann-Whitney U test to compare result among groups.

Result

A-Gross Pathology

In the gross pathology result, all absolute ethanol treated rats showed a hemorrhagic lesion in stomach samples. While, the control negative group rats did not show any gross lesion (Fig. 1). However, the severity of the hemorrhagic lesion was varied depending on groups. Control positive groups rats showed hemorrhagic patches at a size of >5 mm in all rats (Fig. 2). The gross lesion of OME group rats showed a 1-2 mm petechial hemorrhage in all rats (Fig. 3). MG group rats showed a 1-2 mm in size petechial hemorrhage with or without the presence of hemorrhagic patches (Fig. 4). However, the hemorrhagic patches with the presence of petechial hemorrhage were observed in PG group rats (Fig. 5).

Figure 1. Photograph of stomach of control negative group rat

Figure 2. Photograph of stomach of ethanol group rat

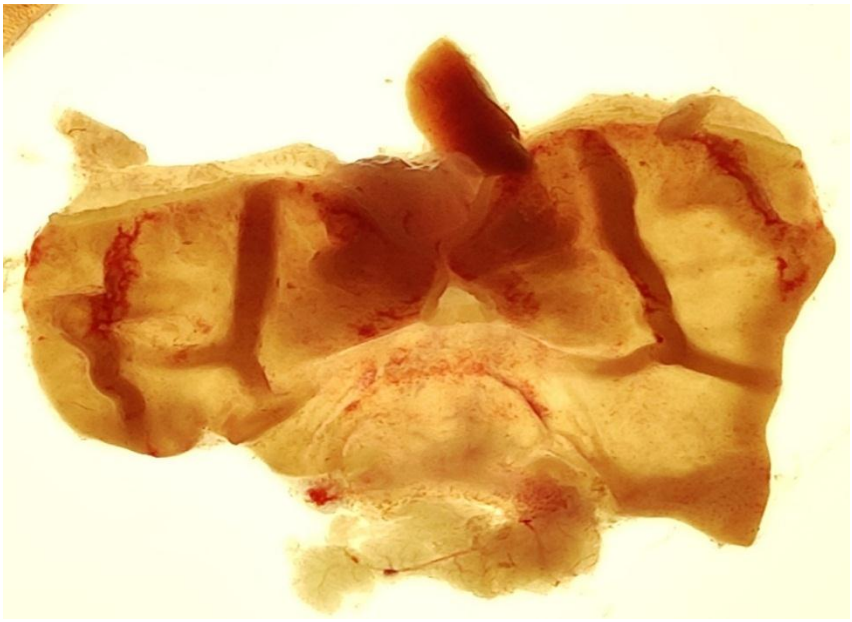


Figure 3. Photograph of stomach of OME group rat

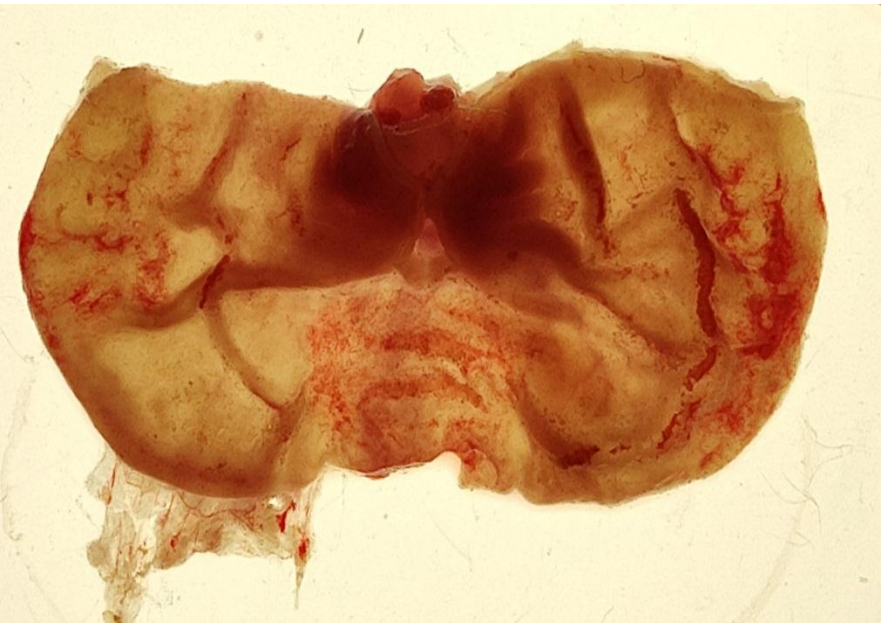


Figure 4. Photograph of stomach of MG group rat

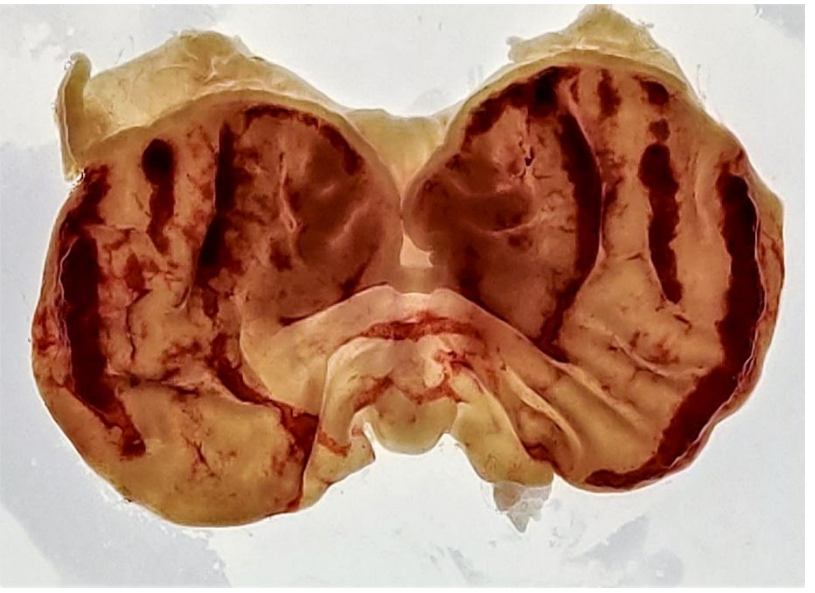


Figure 5. Photograph of stomach of PG group rat

Ulcer index

The ulcer index of alcoholic treated rats shows sever significant differences depending on the group. Ulcer index of control positive was 3.6, OME 1.2, MG 1.8, PG 2 (Table 1& 2).

Table 1
Descriptive means of ulcer index

Groups	Ulcer Index		Protection%
	Mean	±S.D	
C-	0[a]	± 0	0
C+	3.6[b]	± 0.55	100
OME	1.2	± 0.45	66
MG	1.8	± 0.84	50
PG	2	± 0.71	44

Letter a indicate a significant differences between C- group and other groups. Letter b indicate a significant differences between C+ group and groups OME, MG and PG. Letter c indicate a significant between OME group and PG group.

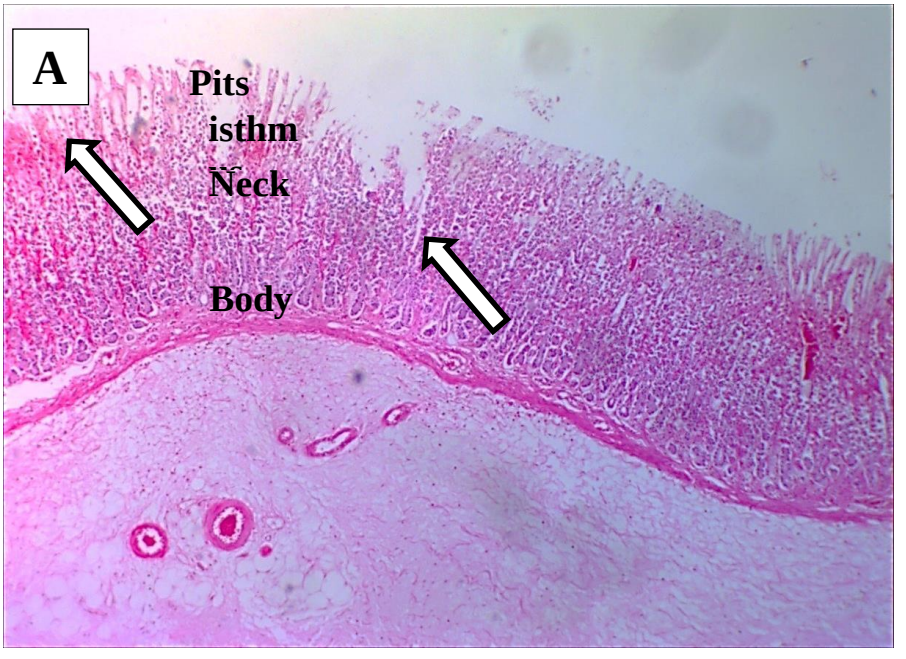
Table 2
Kruskal-wallis ranks of ulcer index

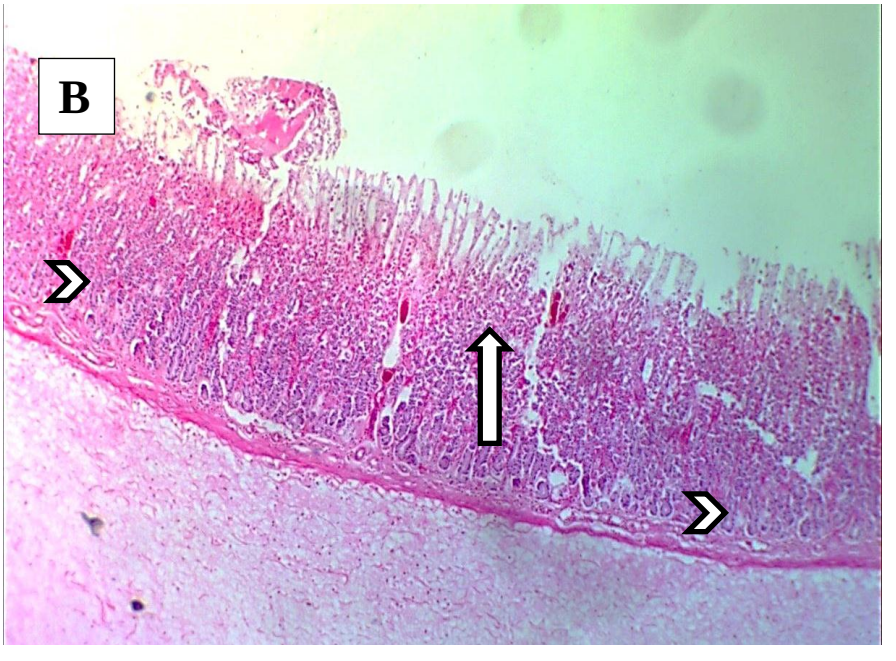
Groups	Ulcer Index Mean Rank
C-	3.00 [a]
C+	22.60[b]
OME	10.30[c]
MG	13.90
PG	15.20

Letter **a** indicate a significant differences between **C-** group and **other groups**. Letter **b** indicate a significant differences between **C+** group and groups **OME**, **MG** and **PG**. Letter **c** indicate a significant between **OME** group and **PG** group.

B-Histological evaluation of gastric lesions

The sever ulcer lesion was observed in control positive rats and these lesion was characterized by severe erosion in gastric mucosa due to necrosis of epithelial cells of pits, isthmus and neck layer of mucosa, also, infiltration of neutrophils cells was observed in mucosa with presence of congestion in surrounding tissue, the hemorrhage found in erosion area and under the erosion area (Fig. 6 a, b& c). In OME group rats the ulcer lesion was manifested with erosion in surface area of mucosa with presence of congestion in the surrounding tissue and presence of hemorrhage in erosion area (Fig. 7 a& b). The ulcer lesion of MG group rats was manifested with erosion in pits area and isthmus involving the partial cells (Fig. 8 a& b). The ulcer lesion of PG group rats was manifested with erosion in pits area and isthmus involving the partial cells (Fig. 9 a& b).


A
Pits
isthm
Ñeck
Body


B

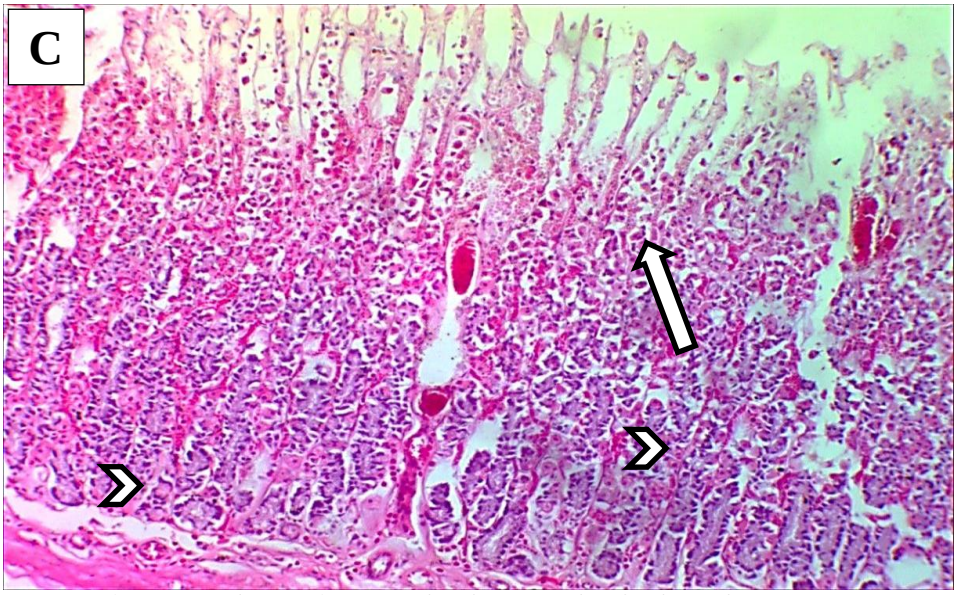
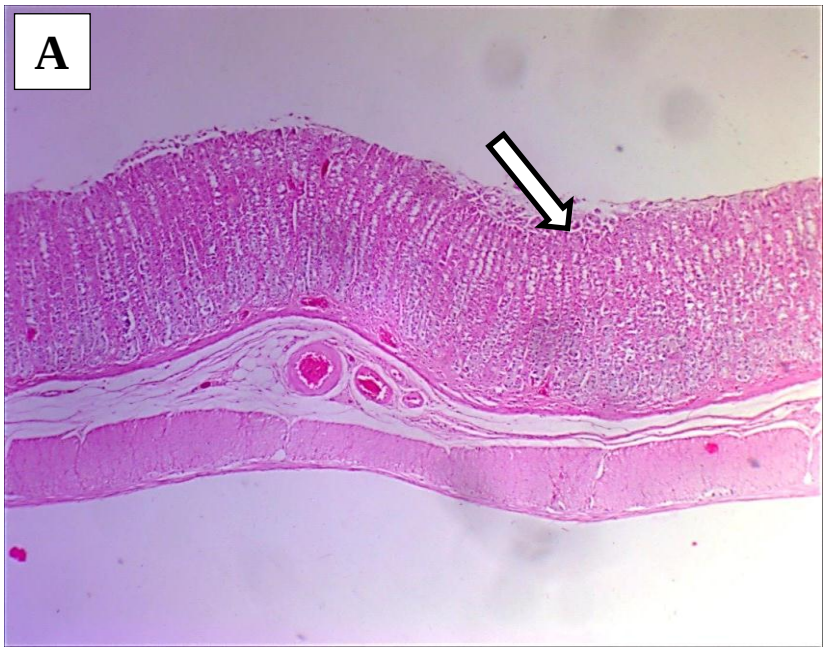


Figure 6. Photomicrograph of stomach of ethanol group rat

Necrosis epithelial cells (arrows) forming spaces in gastric mucosa that involved the pits, isthmus and neck layers, where the frames of pits appeared empty. Also, infiltration of inflammatory cells (especially neutrophil) with presence of hemorrhage (arrowheads) were observed in affected areas. **H&E. A&B: x40 and C: x100.**



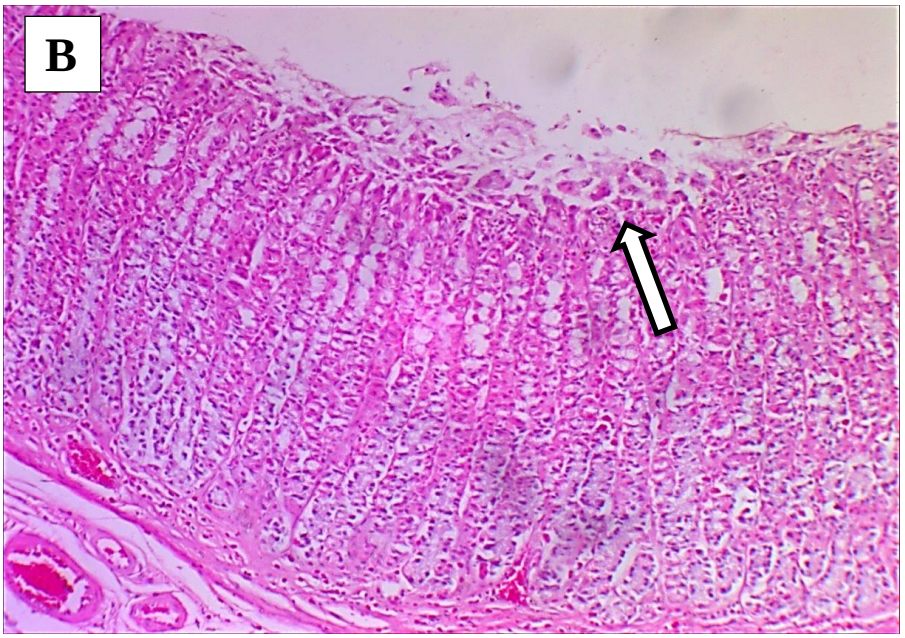
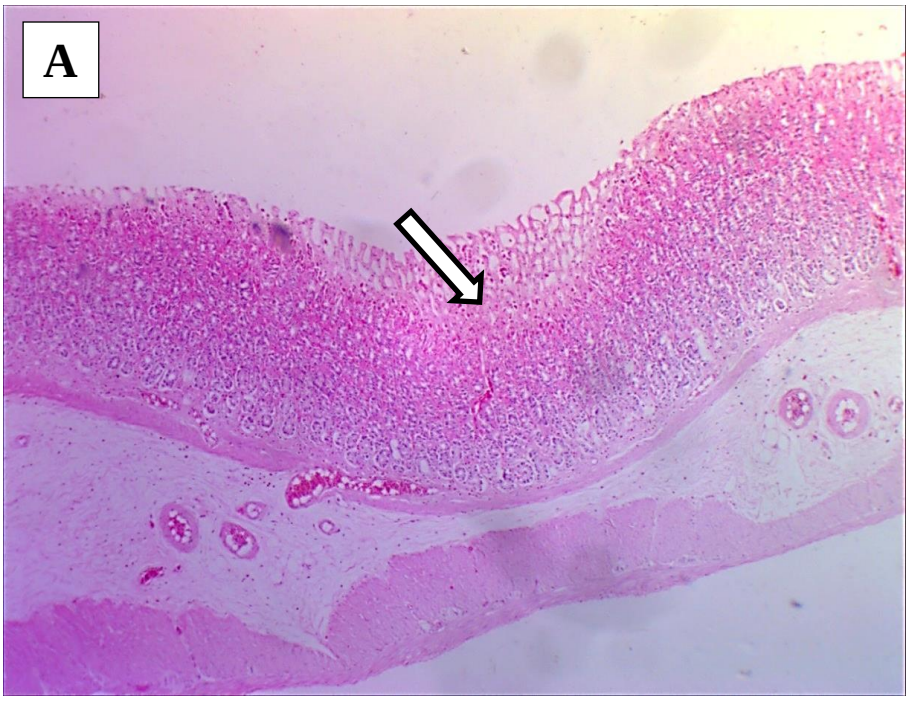


Figure 7. Photomicrograph of stomach of OME group rat

Necrosis of epithelial cells (arrows) was observed in surfaces of gastric mucosa that involving the upper pits layer. **H&E. A: x40 and B: x100**



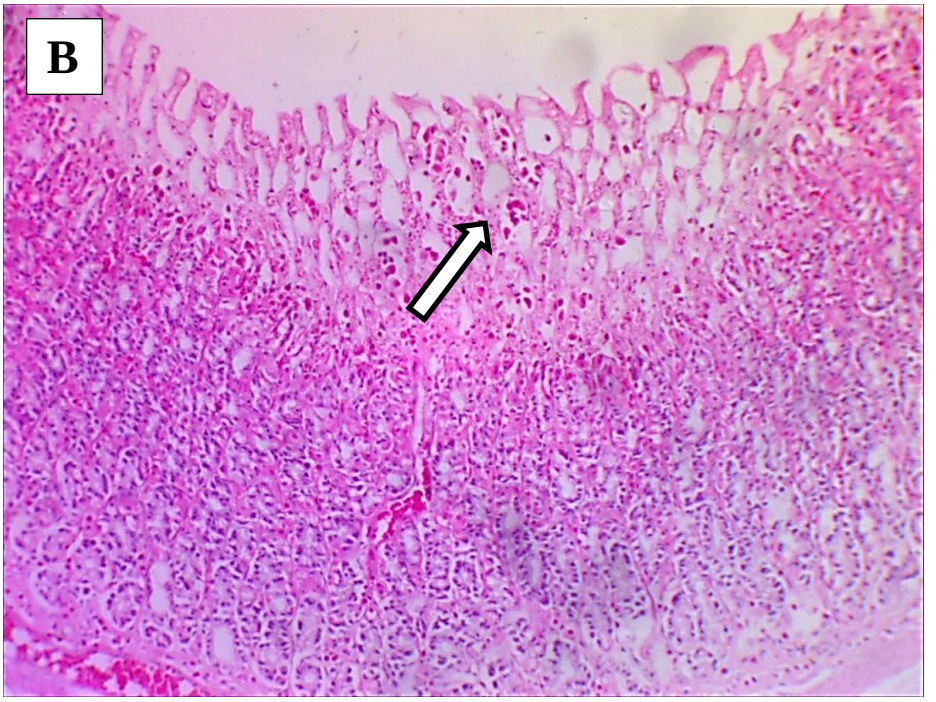
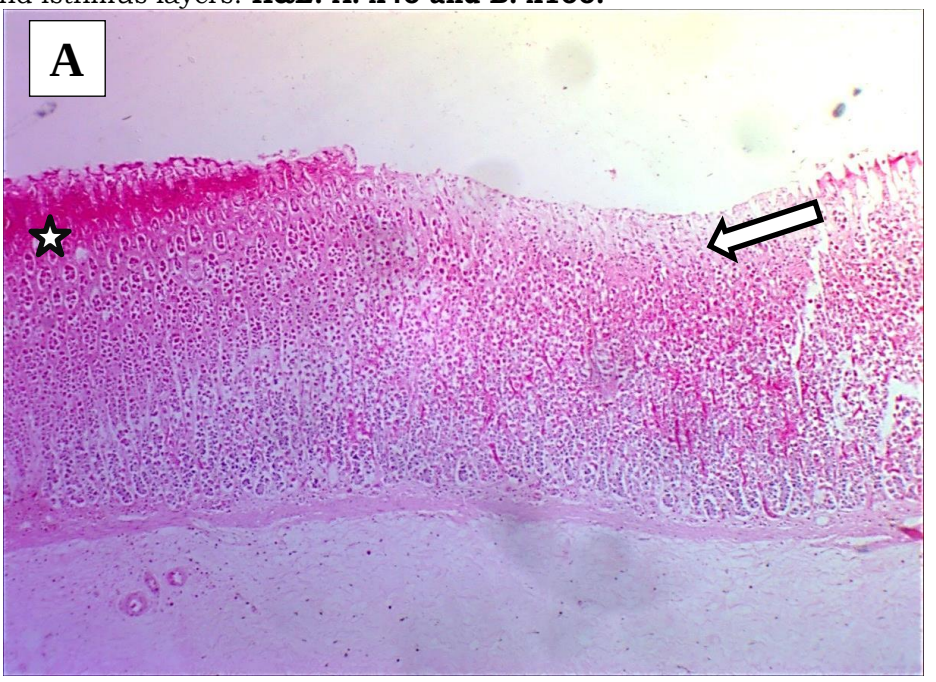


Figure 8. Photomicrograph of stomach of MG group rat

Necrosis of epithelial cells (arrows) was observed in gastric mucosa that involving pits and isthmus layers. **H&E. A: x40 and B: x100.**



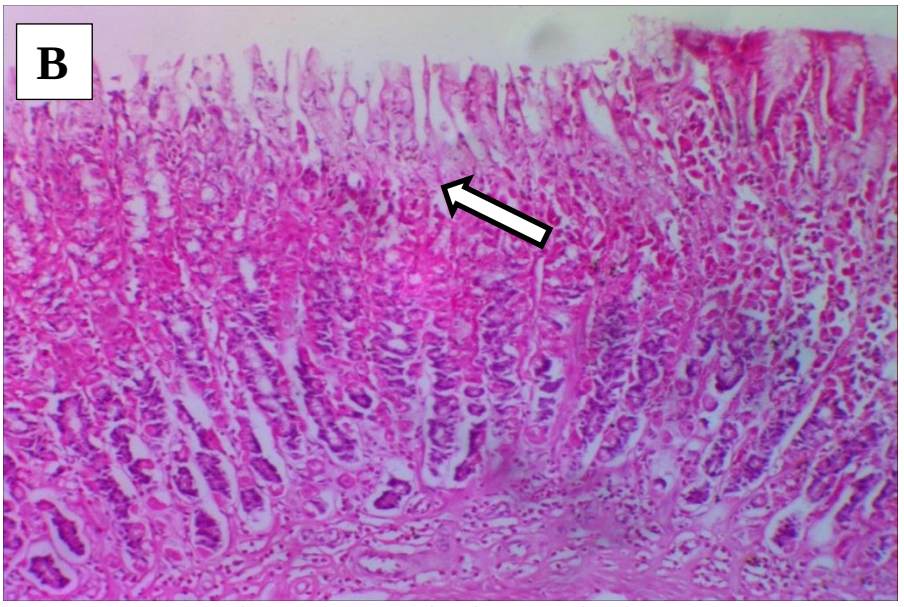


Figure 9. Photomicrograph of stomach of PG group rat

Necrosis of epithelial cells (arrows) was observed in gastric mucosa that involving the pits and isthmus layers. Also, hemorrhage (hemorrhage) was observed in all layers of mucosa. Congestion (stars) was observed in surface of gastric mucosa. **H&E. A: x40 and B: x100.**

Discussion

peptic ulcer disease is a problem of the gastrointestinal tract characterized by mucosal damage secondary to pepsin and gastric acid secretion and can be created experimentally (Koncoro et al., 2015). The ethanol is a usual, convenient model in induction ulcer, it progresses disarrangement in mucosal microcirculation and ischemia, produces free radicals, and releases endothelium (Arawwawala et al., 2010). The current study was conducted to investigate the anti-ulcer activity of the mastic gum (Pistacia Atlantica) and pomegranate peel (Punica Granatum) and to compare between them. The gross pathology result showed a variation in ulcer in ethanol induced ulcer in rats. Where the administration of ethanol orally had the ability to induced ulcer within one hour of induction (Hollander et al., 1985).

However, the ethanol and omeprazole treated rats showed a limited petechial hemorrhage area, where the omeprazole considered the basal antiulcer drugs which have the ability for inhibiting gastric acid secretion in the treatment of peptic ulcer (Novotna et al., 2014). Ethanol and mastic gum treated rats showed petechial hemorrhage in gastric area without the presence of hemorrhagic patches, where as ethanol and pomegranate peel treated rats showed a petechial hemorrhagic patches. The ulcer index of the recent study showed the optimal protection rate was observed in omeprazole and ethanol treated rats compared with other groups. However, the ulcer index result showed a significant protective effect in mastic gum treated rats compare with the pomegranate peel treated rats.

The gross pathology and ulcer index indicated that the mastic gum protection rate was better than pomegranate peel.

In histopathology result the ethanol only treated rats showed destruction in mucosal of stomach that involve all the three layer of pits compare with other groups, where omeprazole and mastic gum treated rats necrosis was involve only the surfaces epithelial cells of pits. However, the pomegranate treated rats showed necrosis in epithelial cells involved the pits and isthmus layer. The histopathology result indicated that the optimum protective effect was observed in omeprazole and mastic treated rats. However, pomegranate peel treated rats showed a less protective effect compare with other.

Conclusion

According to the results of present study, mastic gum and pomegranate peels showed preventive effects against ethanol to induce ulcer in male rats compared with ethanol only treatment. However, mastic gum showed more efficient preventive effects than pomegranate peels. Also, omeprazole as a common anti-ulcer drug showed efficient preventive ulcer induction more than pomegranate peels.

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