Gastroprotective activity of alpha-pinene in ethanol-induced gastric mucosal damage in rats

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ABSTRACT
Gastric ulcer is the most common disorder of the gastrointestinal tract, it occurs mainly in the stomach. α-pinene is a bicyclic terpene and its oil extracted from Lamiaceae, conifers, citrus and other plants. It has broad pharmacological activities. This study was done to assess the anti-ulcer activity of α-pinene against gastric ulcer in rat. This study included 6 groups with 6 rats in each group. Group 1 is healthy group and group 2 is control group (untreated ulcer group). Group 3 (positive group) was orally pretreated with 20 mg/kg ranitidine. Groups 4, 5, and 6 (experimental groups) were orally pre-treated with α-pinene at 500, 1000 and 1500 mg/kg doses, respectively. Result of this study appeared that the ulcer control group showed severe mucosal injury, while pre-treatment with α-pinene resulted in significantly ($P<0.05$) protection of gastric mucosal injury, good neutralizing properties, and increase in mucus production in comparison to ranitidine pretreated and ulcer control groups. In conclusions, the present results suggest that α-pinene exhibit an anti-ulcer activity against ethanol-induced gastric ulcer in rats.

دراسة فعالية الالفا-باينين في حماية المعدة ضد الكحول المسبب للقرحة المعدية

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الكلمات المفتاحية: الالفا-باينين، الايثانول، قرحة المعدة، رانتدين، جرذان.

الخلاصة
قرحة المعدة تعد من اكثر اضطرابات الجهاز الهضمي شيوعا، وتحدث في المعدة. ويعتبر الالفا-باينين وهو من مجموعة التربينات ثنائية الحلقة، واشهر مكونات الزيوت المستخلصة من Lamiaceae، صنوبر، الحمضيات وغيرها من النباتات وهو ذو تأثير دوائي واسع الطيف. الهدف من هذه الدراسة هو تقييم فعالية الألفاباينين كمعالج للقرحة المحدثة بكحول الايثانول. هذه الدراسة تتضمن ستة مجاميع، كل مجموع تحتوي 6 جرذان.المجموعة الاولى هي المجموعة الصحية والمجموعة الثانية هي مجموعة السيطرة (المصابة بلقرحة). المجموعة الثالثة وتشمل الجرذان التي جرعت بالرانتدين (20 ملغم/كغم) قبل احداث القرحة المعدية فيها، المجاميع (4، 5، و6) وتشمل الجرذان التي جرعت ب (500، 1000، 1500 ملغم/ كغم على التوالي) من الالفا-باينين قبل احداث القرحة المعدية فيها، بعد مرور ساعة، كل الجرذان تجرع بكحول الايثانول (ماعدا المجموعة الاولى) لاحداث القرحة المعدية. بشكل اجمالي، أظهرت المجموعة الثانية قرحة معدية حادة، بينما المجاميع المجرعت بالالفاباينين قبل الاصابة أظهرت حماية ملحوظ ($P<0.05$) بتقليل القرحة المعدية، معادلة حموضة المعدة، وزيادة في انتاج بطانة المعدة المخاطية بالمقارنة مع المجموعة الثانية والثالثة. من ذلك نستنتج ان الالفاباينين يمتلك نشاطاً ضد القرحة المعدية المحدثة بواسطة كحول الايثانول في الجرذان.

1. INTRODUCTION

Gastric ulcer is a common disorder of the gastrointestinal tract, it occurs mainly in the stomach (1). Each year gastrointestinal ulcer affects 4 million people around the world (2). Gastric ulcer was more frequent in patients ≥ 60 years of age (68%) than < 60 years of age (32%) and about 84% of this population are smokers (3). The general physiopathology

of gastric ulcer due to excessive production of some endogenous aggressive factors including HCL, pepsin, refluxed bile, leukotriene, reactive oxygen species (ROS) and reduce gastroprotective factors, which include the mucus-bicarbonate barrier, prostaglandins (PG), surface active phospholipids, cell renewal and migration, mucosal blood flow, non-enzymatic and enzymatic antioxidants and many growth factors (4,5). Absolute alcohol has been approved to induce gastric mucosal damage in animals. Pure ethanol is lipid soluble and cause severe mucosal damage.

Ethanol at low concentrations (5%) may strongly stimulate gastric acid secretion and thereafter causing mucosal damage. Terpenes are a group of molecules that mainly consist from 10 - 15 carbon atoms built from isoprene which is composed from 5 carbon building blocks. Monoterpenes, sesquiterpenes, and diterpenes are abundant in the essential oils of plants (6). α-pinene is a bicyclic terpene and its oil extracted from Lamiaceae, conifers, citrus and other plants (7). It is found in many pharmaceutical preparations as solvent and flavor agents and as therapeutic preparation like Rowatinex and Rawachol. It has broad pharmacological effects as antibacterial (8), antifungal (9), insecticide (10), anticancer (11), and neurological effects (12). The aim of the present study is to evaluate the gastroprotective activity of α-pinene against ethanol-induced gastric ulcer in rats.

2. MATERIALS AND METHODS

Induction of Gastric ulcer by ethanol in rats:

Gastric ulcer was induced by absolute ethanol (5 ml/kg) given by orogastric intubation according to the method described by De Pasquale et al (1995) [13]. All groups fasted for 2 days before the experiment [14], and allow them for free access drinking water up to 2 h before the experiment.

Experimental design:

36 rats with body weights (150 – 265g) having said criteria were selected. 6 rats were categorized into healthy, 6 rats were categorized into gastric ulcer control and 18 rats were categorized into experimental groups which were treated with alpha-pinene and other 6 rats categorized into positive controls were received oral doses of 20 mg/kg ranitidine in 0.03ml of D.W.

Animal group:

- Control (non-ulcer) group, this group received D.W. only.
- Ulcer group was orally administered with vehicle D. W. and corn oil.
- Positive controls were received oral doses of 20 mg/kg ranitidine in 0.03ml of D.W.
- Group (4, 5, and 6) (Alpha-pinene treated group) were received oral dose of 500mg (0.58 ml), 1000mg (1.16 ml), 1500mg (1.74 ml) /kg respectively of alpha-pinene (Sigma, USA) in 2.25 ml/kg corn oil.

After one hour of this pre-treatment; all groups (except the control group) of rats were gavaged with absolute ethanol (5 ml/kg). After one hour, the rats were sacrificed by cervical dislocation using diethyl ether in overdose as anesthesia (15). The stomachs excised immediately and rapidly immersed in 10% buffered formalin solution.

Assessment of sectional gastric lesions:

For the presence and severity of ulcerative lesions, the gastric surface was assessed. These lesions were measured with magnifying glass (10X amplification) containing millimeteric ruler and expressed as ulcer index (UI) in millimeters (mm) and percentage of ulcer inhibition. The UI was calculated by the summation of each stomach lesion lengths. The widths of superficial ulcers were < 1 mm, multiplied by 1; those within range 1 - 2 mm multiplied by 2; and the deeper lesion > 2 mm multiplied by 3. In addition, each 5 spot lesions were viewed as equivalent to ulcer length of 1 mm.

The percentage of ulcer inhibition was calculated as follows:

Inhibition of ulcer (%) = [(control UI-test UI)/ Control UI] ×100 (16).

Measurement the acidity of gastric juice (PH):

Each stomach was opened along the greater curvature. The acidity of gastric contents was separated by centrifugation at 3500 rpm for 30 min. The supernatant was taken for the determination of gastric pH with aiding of digital pH meter (17).

Assessment of gastric mucus:

The method used for the assessment of gastric mucus described by Corne, et al. (1974). The absorbance of the colored solution was measured on a spectrophotometer (Hitachi U-3210, Tokyo, Japan) at 605 nm. The amount of alcian blue eluted from the tissue was analyzed against a standard curve which was plotted by known graded concentrations (5–50 mg/L) of alcian blue solutions versus them absorbance. The results were expressed as an mg alcian blue/g wet tissue (18).

Histological evaluation of gastric lesions:

The tissue specimen was taken and kept in formalin 10%. This specimen was cut accurately and experimentally. Blocks were then dehydrated in a series of alcohols, cleared by xylene and embedded in paraffin. The section of 7 mm thickness were obtained and stained by hematoxylen and eosin as common histological method (11). Each section was investigated under a microscope (Humason, Gretchen).

Ethics:

The study was approved by the ethics Committee for animal experimentation, collage of pharmacy, Karbala University, Iraq. During the experiments, all animals deal with human care according to the criteria mentioned in the "Guide for the Care and Use of Laboratory Animals" made by the Sciences National Academy and published by the Health National Institute.

Statistical analyses:

Statistical analyses were achieved using SPSS 19.0 for windows. Inc. The data were showed as means ± SEM and statistically analyzed by one-way analysis of variance (ANOVA) followed by LSD post-hoc test and Chi-square for non-quantitated data. P values < 0.05 were considered significant.

3. RESULTS

Sectional assessment of gastric lesions:

The gastroprotective activity of α-pinene in gastric ulcer model is explained in Table 1. The results showed that α-pinene pre-treated rats before being administrated absolute ethanol had significantly reduced gastric ulcer lesions formation compared to D.W. and corn oil pre-treated rats (ulcer group). It was also observed that gastric mucosa protection was more predominant in 1000 and 1500 mg/kg α-pinene pre-treated rats which have approximately the same activity (Table 1). Although, mucosal damage was significantly induced by ethanol, α-pinene reduced the size and severity of this damage in dose dependently manner. The gastric ulcer inhibition was significantly observed in α-pinene pre-treated rats in comparison to ranitidine pre-treated group.

Assessment of gastric mucus and acidity of gastric juice (PH):

The results showed that the acidity of gastric juice is significantly neutralized by α-pinene in pre-treatment rats in comparison to healthy and ulcer groups (Table 2). There is no significant difference in PH value of α-pinene pre-treated groups; also there is no significant difference in the potency of neutralized properties of both ranitidine and α-pinene (Table 2).

The results showed that the production of mucus protective layer is significantly increase in α-pinene pre-treated rats in comparison to healthy and ulcer groups. There is no significant difference between different doses (500, 1000, 1500 mg/kg) of α-pinene in mucus production, The results showed that the production of mucus protective layer is significantly increase in α-pinene pre-treated rats in comparison to ranitidine pretreated rats (Table 2).

Histological evaluation of gastric lesions:

Histological view of gastric lesions induced by ethanol in ulcer group pre-treated with vehicle (D.W. & corn oil), showed comparatively extensive gastric mucosal damage, sloughing of the epithelial mucosa, congestion of blood vessel and leucocytes infiltration of both mucosal and submucosal layer (Figure 3). Ranitidine pre-treated rats showed a mild gastroprotective activity with degenerative change in the mucosa with cystic dilation of gastric gland (Figure 4). The α-pinene pre-treated rats had comparatively good gastric mucosa protection as demonstrated by falling-off in ulcer area, decrease or absence of the epithelial mucosal sloughing, congestion of blood vessel and leucocytes infiltration (Figure 5, 6, and 7). α-pinene has been shown a dose-dependent cytoprotective effects.

Table (1): Mean ulcer index and ulcer inhibition% of the five experimental groups (vehicles (controls), ranitidine, and α-pinene (500, 1000, 1500)) during period of experiment (N=6 in each group).

Treatment	Dose (mg/kg)	Ulcer index (mm^2) Mean±SD	Ulcer inhibition % Mean±SD
Control (D.W. & corn oil)	0.03ml+2.25ml/kg	938.33±26.10	-
Ranitidine	20	144.67±50.82*	84.58±5.42
α-pinene	500	62.72±8.00**	93.32±0.85
α-pinene	1000	6.95±1.84**	99.26±0.20
α-pinene	1500	6.93±1.86**	99.26±0.20

*: $P<0.05$, significant vs. control group.

**: $P<0.05$, significant vs. control and ranitidine pretreated groups.

Table (2): Mean mucus production and PH value of the six experimental groups (healthy, control, ranitidine, and α-pinene (500, 1000, 1500)) during period of experiment (N=6 in each group).

Treatment	Dose (mg/kg)	Mucus production Mean±SD	PH Mean±SD
Health (D.W.)	5ml/kg	20.59±1.14	1.92±0.31
Control (D.W. & corn oil)	0.03ml+2.25ml/kg	10.84±0.84*	3.30±0.62
Ranitidine	20	29.65±15.94	7.03±0.49
α-pinene	500	48.18±4.55**	7.00±0.90
α-pinene	1000	56.28±10.43**	7.00±0.35
α-pinene	1500	50.36±10.23**	6.54±0.22

*: $P<0.05$%, significant vs. healthy, ranitidine, and α-pinene pretreated groups.

**: $P<0.05$%, significant vs. control ant ranitidine pretreated groups.

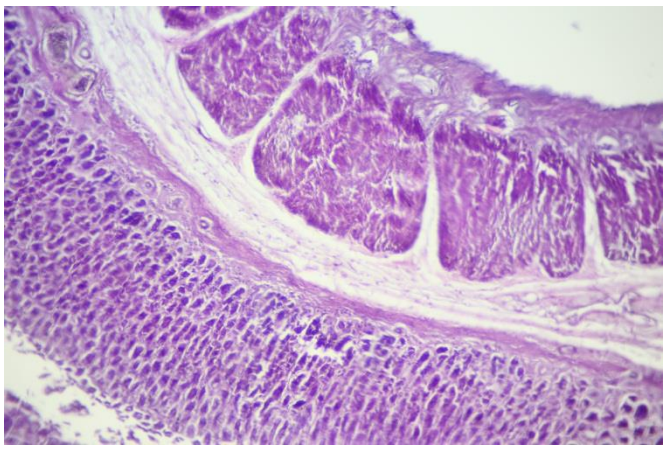

Figure (2): Histological section of gastric mucosa in normal rat at (10X magnifications). There is normal epithelial tissue.

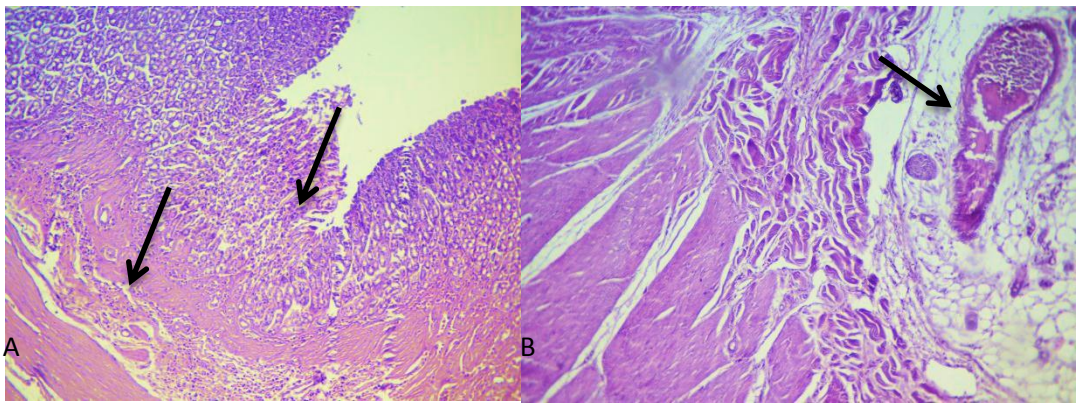

Figure (3): Histological section of gastric mucosa in a rat pre-treated with D.W. and corn oil (ulcer control) at (10 X magnification). A&B pictures show severe disruption to the surface epithelium, and congestion of the blood vessels of both mucosa and submucosal layer with leucocytes infiltration (Arrows).

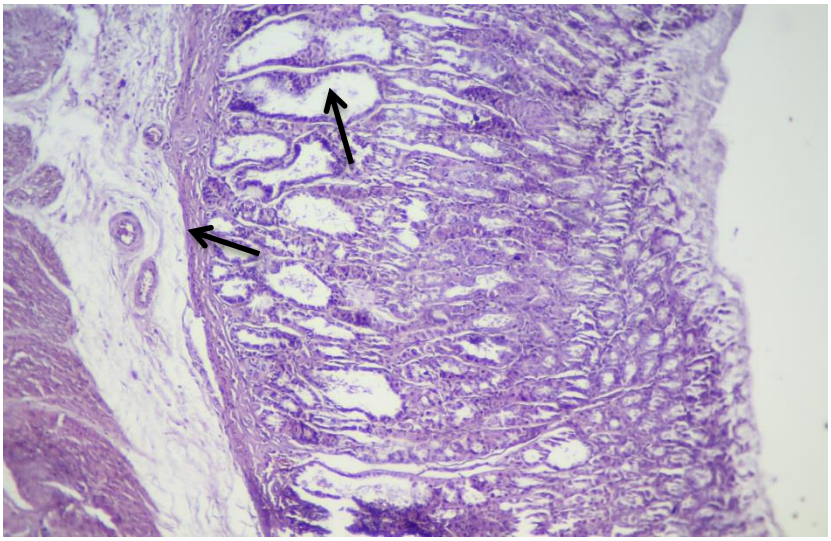

Figure (4): Histological section of gastric mucosa in a rat pre-treated with ranitidine at (10 X magnifications). There is degenerative change in the mucosa with cystic dilation of gastric gland and congestion of blood vessels (Arrows).

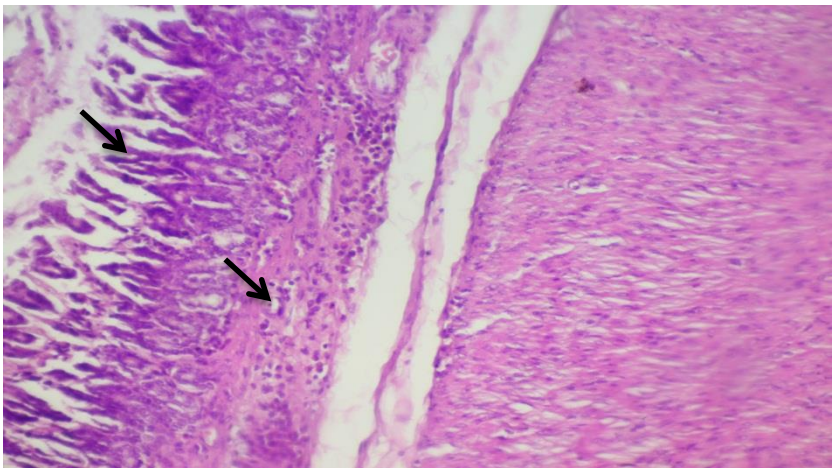

Figure (5): Histological section of gastric mucosa in a rat pre-treated with α-pinene (500 mg/Kg). There is mild sloughing of epithelial of mucosa and infiltration of inflammatory cell like lymphocytes and eosinophilia (arrows) (10 X magnifications).

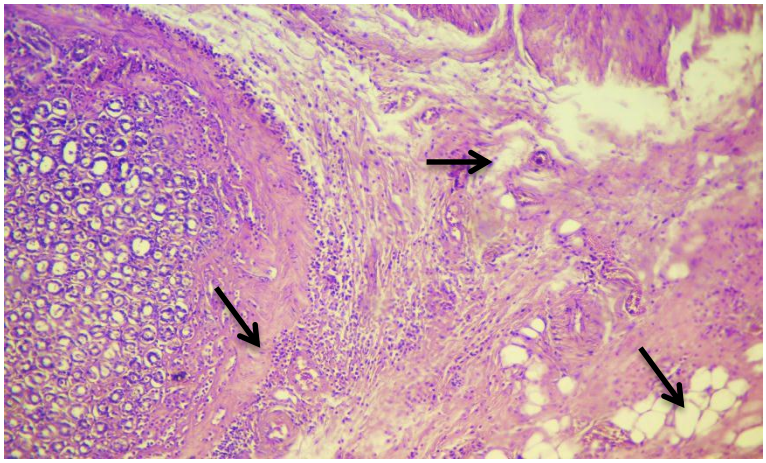

Figure (6): Histological section of gastric mucosa in a rat pre-treated with α-pinene (1000 mg/Kg) at (10 X magnifications). There are fewer of cystic dilation gastric glands with inflammatory cells in mucosa and submucosa and congestion of blood vessels. (Arrows)

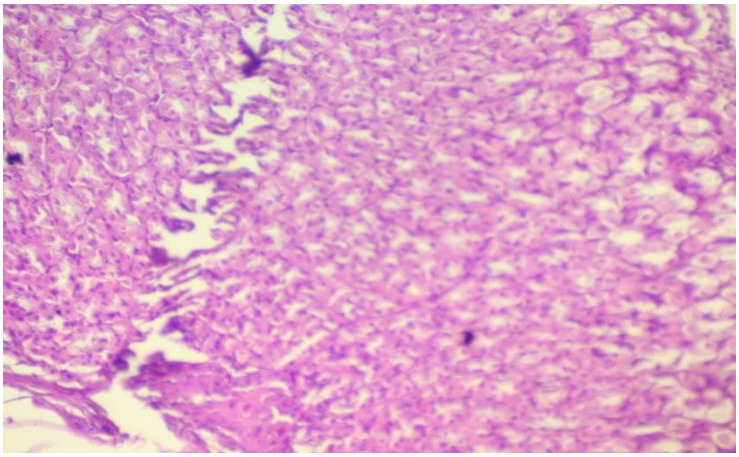

Figure (7): Histological section of gastric mucosa in a rat pre-treated with α-pinene (1500 mg/Kg) at (10 X magnifications). There are only mild degenerative changes of epithelial of mucosa (cloudy and swelling).

4. DISSCUSION

Although, there are many products available in the market as antiulcer agents, including antacids; histamine H2-blockers, and proton pump inhibitors, most of them produce many adverse effects, such as hematopoietic changes, thrombocytopenia anaphylaxis reactions, gynecomastia, , acute interstitial nephritis, hepatotoxicity and nephrotoxicity. Therefore, there is a need to search for new less expensive antiulcer agents with minor side effects. In this line, medicinal plants are the most beneficial sources of new drugs, and have been given good results in the treatment of gastric ulcers. When absolute ethanol was administrated orally, it is considered aggressive factor to the stomach because it disrupts the gastric mucosal barrier and induces immediately microvascular changes (19). The typical characteristics of alcohol injury after oral administration are linear hemorrhagic

lesions, mucosal friability, wider submucosal edema, inflammatory cells infiltration, and epithelial cell degeneration in the stomach (20).

In this study, α-pinene exhibit significant gastroprotective activity with very high percentage of ulcer inhibition in comparison to ranitidine and ulcer groups. Valim Araujo et al. (2011) demonstrated that Protium heptaphyllum extract (containing 40% α-pinene) exerts its gastroprotective activity by increasing cyclooxygenase-2 (COX-2) and epidermal growth factor (EGF) expression and may be due to its possible antioxidant property (21). Souza et al. (2011) mention that oral administration of α-terpineol dose-dependently reduced gastric lesions at all doses tested (16).

In this study, α-pinene demonstrated its antacid properties by significant increase pH in compare with healthy and ulcer group which may suggest that its gastroprotective action may be produce by reduce gastric acid secretion. Also, it is significantly increase the mucus production at all dose in compare with ranitidine pretreatment and control group. Rozza et al. (2013) mention that experimentally a huge number of aromatic essential oils and medicinal plants have gastroprotective and ulcer healing properties by reduce gastric volume and acidity and enhance mucus production (22). Al-Radahe et al. (2012) demonstrated that the Swietenia mahagoni leaf extract (containing many types of terpenes) can inhibit gastric lesions formed by ethanol and mention that leaf extract is significant increase in mucus production suggests that the gastric mucosal strengthening mechanism contributes to the anti-irritant potential of the Swietenia mahagoni (23).

In the present study, we observed the gastric mucosal protection and fall-off in ulcer area, decrease or absence sloughing of the epithelial mucosa, congestion of blood vessel and leucocytes infiltration in α-pinene pretreated rats. Kobayashi et al. (2001) mention that teprenone (monoterpene) produced a gastric protective effect through inhibition of neutrophil infiltration in the ulcerated gastric tissue (24). While, Shimizu et al. (2000) reported that the healing of gastric ulcers can be promoted by reduction of neutrophil infiltration into ulcerated gastric tissue in rats (25).

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