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RESEARCH ARTICLE **HISTOLOGY**

Potential Therapeutic Effects of *Juniperus phoenicea* Extract Against Ethanol-Induced Gastric Injury in Male Mice: Histopathological and Ultrastructural Evidence

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ABSTRACT

This study investigated the potential therapeutic effect of the aqueous extract of *Juniperus phoenicea* against ethanol-induced gastric injury in male Swiss albino mice. Sixty adult mice were randomly allocated into four groups: control, ethanol-treated, extract-treated, and ethanol + extract-treated groups. Gastric injury was induced by oral administration of absolute ethanol (1 mL/kg) on alternate days, while the plant extract was administered orally at a dose of 40 mg/kg daily for seven consecutive days. Ethanol exposure caused marked gastric damage characterized by epithelial erosion, vascular congestion, inflammatory cell infiltration, and disruption of normal mucosal architecture. In addition, ethanol-treated animals showed reduced body weight gain and alterations in hematological parameters. In contrast, treatment with *J. phoenicea* extract significantly improved gastric tissue structure, reduced inflammatory changes, and promoted restoration of epithelial organization. The treated group also demonstrated improvement in body weight and partial normalization of hematological indices. Ultrastructural examination confirmed recovery of cellular integrity, including restoration of mitochondria, secretory granules, and rough endoplasmic reticulum in gastric epithelial cells. These findings suggest that the aqueous extract of *J. phoenicea* possesses promising therapeutic activity against ethanol-induced gastric injury, possibly through antioxidant, anti-inflammatory, and tissue regenerative mechanisms. Further studies are recommended to identify its active constituents and evaluate its clinical applicability.

الفعالية العلاجية لمستخلص نبات العرعر الفينيقي في نموذج فأر مصاب بإصابة معدية ناتجة عن الإيثانول: أدلة خلوية، ونسيجية مرضية، وفوق بنيوية

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الكلمات المفتاحية

العرعر الفينيقي
الإيثانول
قرحة المعدة
التأثير العلاجي
علم الخلايا
علم الأنسجة المرضية

الملخص

هدفت هذه الدراسة إلى تقييم التأثير العلاجي المحتمل للمستخلص المائي لنبات العرعر الفينيقي (*Juniperus phoenicea*) ضد الإصابة المعدية الناتجة عن الإيثانول في ذكور الفئران البيضاء السويسرية. تم توزيع ستين فأرًا بالغًا عشوائيًا إلى أربع مجموعات: مجموعة ضابطة، ومجموعة معالجة بالإيثانول، ومجموعة معالجة بالمستخلص، ومجموعة معالجة بالإيثانول مع المستخلص. أُحدثت الإصابة المعدية عن طريق إعطاء الإيثانول المطلق فمويًا بجرعة (1 مل/كغ) يومًا بعد يوم، في حين أُعطي المستخلص النباتي فمويًا بجرعة (40 ملغ/كغ) يوميًا لمدة سبعة أيام متتالية. أدى التعرض للإيثانول إلى حدوث تلف واضح في المعدة تمثل في تآكل الظهارة، واحتقان الأوعية الدموية، وارتشاح الخلايا الالتهابية، واضطراب البنية الطبيعية للغشاء المخاطي. كما أظهرت الحيوانات المعالجة بالإيثانول انخفاضًا في زيادة وزن الجسم وحدوث تغيرات في المؤشرات الدموية. وفي المقابل، أدى العلاج بمستخلص العرعر الفينيقي إلى تحسن ملحوظ في تركيب نسيج المعدة، وانخفاض التغيرات الالتهابية، واستعادة التنظيم الطبيعي للخلايا الظهارية. كما أظهرت المجموعة المعالجة تحسنًا في وزن الجسم واستعادة جزئية للمؤشرات الدموية. وأكد الفحص بالمجهر الإلكتروني تحسن سلامة الخلايا، بما في ذلك استعادة الميتوكوندريا، والحبيبات الإفرازية، والشبكة الإندوبلازمية الخشنة في الخلايا الظهارية للمعدة. وتشير هذه النتائج إلى أن المستخلص المائي لنبات العرعر الفينيقي يمتلك نشاطًا علاجيًا واعدًا ضد الإصابة المعدية الناتجة عن الإيثانول، وربما يعود ذلك إلى خصائصه المضادة للأكسدة، والمضادة للالتهاب، والمحَفِّزة لتجدد الأنسجة. ويوصى بإجراء المزيد من الدراسات لتحديد مكوناته الفعالة وتقييم إمكانية تطبيقه سريريًا.

Introduction

Over recent decades, considerable progress has been made in understanding the biological and ecological characteristics of the *J. phoenicea* complex through multidisciplinary studies involving taxonomy, biogeography, morphology, biochemistry, genetics, ecology, and physiology [1,2]. The

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genus *Juniperus* comprises nearly 70 species distributed mainly in the Northern Hemisphere [3]. Among them, *J. phoenicea* is a dominant evergreen species in the Al-Jabel Al-Akhdar region, representing nearly 80% of the local tree and shrub flora [4], and is widely distributed across the Mediterranean rocky habitats [5].

This species is rich in bioactive compounds with reported antiproliferative and medicinal properties. In traditional medicine, *juniper* extracts have been used to relieve gastrointestinal disorders such as bloating, discomfort, and poor appetite [6]. Various *Juniperus* species have also been employed as antihelmintic, antiseptic, and wound-healing remedies [7]. Increasing scientific evidence further supports the pharmacological importance of *J. phoenicea* as a promising source of therapeutic agents [8].

Previous experimental studies demonstrated that the essential oil of *J. phoenicea* showed protective activity against HCl/ethanol-induced gastric ulceration, reducing ulcer severity, enhancing mucus secretion, and preserving epithelial integrity, effects largely attributed to its antioxidant properties [9]. Histological studies further confirmed reduced epithelial erosion and inflammatory infiltration, with improved tissue organization. In addition, *J. phoenicea* may promote tissue repair through enhanced collagen formation, epithelial regeneration, and structural reinforcement [10].

Excessive or chronic ethanol intake is a major cause of gastric mucosal injury, leading to erosion, ulceration, and bleeding [11]. Ethanol also induces inflammatory responses through macrophage polarization and increased Th17 differentiation, resulting in elevated TNF-α and IL-17 levels and impaired mucosal barrier integrity [12,13]. Moreover, ethanol promotes oxidative stress by generating free radicals and reducing antioxidant defenses, thereby aggravating tissue damage and delaying healing [14].

Although conventional anti-ulcer therapies such as quadruple regimens with bismuth compounds, antibiotics, and proton pump inhibitors are available, their use may be limited by recurrence, adverse effects, and bacterial resistance [15]. Therefore, safer multi-target alternatives capable of reducing inflammation, oxidative stress, and tissue injury are needed [16]. Accordingly, the present study aimed to evaluate the therapeutic potential of *J. phoenicea* against ethanol-induced gastric injury, with emphasis on its protective and healing effects.

Methodology

Preparation of the aqueous extract of *J.phoenicea:* The aqueous extract of *J. phoenicea* was prepared using dried leaves that were ground before use. Briefly, 0.5 g of powdered dried leaves was added to 50 mL of distilled water and boiled at 100°C for 1 min. The container was immediately covered and allowed to cool at room temperature to facilitate the extraction of the active constituents. The mixture was then filtered through sterile gauze to remove plant residues and obtain a clear solution. The extract was freshly prepared before administration to ensure consistency and minimize degradation.

It was given orally at 0.1 mL per mouse, which is equivalent to 40 mg/kg body weight. Although the method of preparation of the extract is consistent with traditional aqueous extract methods, the dosage was based on previously published experimental protocols. [17]. Absolute ethanol was obtained from Sigma (Germany) and administered orally at a dose of 1 mL/kg, a concentration previously reported to reliably induce gastric lesions in experimental animals [18].

Experimental animals and treatment: Sixty healthy adult male Swiss albino mice (Mus musculus), weighing between 20-30 g, 8-10 weeks old, were maintained under controlled conditions, providing 12 hours light and dark periods, temperature 22 ± 4 °C, and free access to standard diet and water [19]. The animals were divided randomly into four groups, i.e., 15 mice in each group. The control group was given distilled water orally at a dose rate of 0.2 mL/mouse/day for seven consecutive days. For the induction of gastric injury, the mice of the ethanol group were fasted overnight, given absolute ethanol orally at a dose rate of 1 mL/kg body weight on alternate days for seven days, as previously described by our group [18]. Extract – *J. phoenicea* aqueous extract was given orally at the dose rate of 40 mg/kg/day for 7 days, as previously described by our group [17]. Ethanol + Extract – ethanol was given as above, along with the extract once daily for 7 days.

Sample collection and analyses: After the completion of the experimental protocol, all sixty mice were euthanized humanely, and the blood and gastric tissue samples were collected for further studies. For the hematological studies, the blood samples were randomly collected from fifteen mice in each group (n=15). The gastric tissue samples were used to study the histopathology and ultrastructural changes.

Body weight assessment: Body weights of all experimental animals were recorded at the start and at the conclusion of the study, with additional weekly measurements obtained using a calibrated electronic balance. Changes in body weight and percentage weight gain were calculated according to established methods [20].

Hematological evaluation: Twenty-four hours after the termination of the experimental period, unanaesthetized mice from the control as well as the treated groups were sacrificed by cervical dislocation, and blood was collected from the cervical vessels into sterile tubes containing ethylenediaminetetraacetic acid as an anticoagulant. Parameters such as the concentration of hemoglobin (Hb), red blood cell count (RBCs), packed cell volume (PCV) , mean corpuscular volume (MCV) , mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total count of white blood cells (WBCs), and platelet count were carried out using an automated hematology analyzer DLAGON Cell 60.

Histology: Samples from the glandular region of the stomach were fixed in 10% neutral buffered formalin and Bouin's solution, dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin wax. Tissue sections (5 µm) were prepared using a rotary microtome (Leica RM 2125) and stained with hematoxylin and eosin (H&E) and Altman's stain according to standard methods [21]. Slides were examined and photographed using a Nikon Eclipse E400 microscope equipped with a digital camera.

Transmission electron microscopy (TEM): For ultrastructural analysis, small fragments of freshly excised gastric tissue were immediately immersed in 2% glutaraldehyde prepared in 0.1 M phosphate buffer (pH 7.3) and fixed for approximately two hours at 4 °C. The specimens were subsequently transferred to 4F1G fixative (formalin–glutaraldehyde mixture) in the same buffer for an additional 24 hours. Post-fixation was carried out using 2% osmium tetroxide (OsO_4) at 4 °C for two hours. Following fixation, the tissues were dehydrated through a graded ethanol series and embedded in an Epon–Araldite resin mixture using labeled beam capsules. Ultrathin sections (approximately 50 nm in thickness) were obtained with an LKB ultramicrotome and mounted on 200-mesh uncoated

copper grids. The grids were contrasted by double staining with uranyl acetate for 30 minutes and lead citrate for 20–30 minutes. Ultrastructural examination was performed using a JEOL 100 CX transmission electron microscope.

Statistical analysis: Data are presented as mean ± standard error of the mean (SEM). Statistical comparisons among groups were conducted using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test. Differences were considered statistically significant at $p < 0.05$.

Ethical approval: All experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee of Omar El-Mukhtar University, Libya, and all procedures were carried out in accordance with internationally accepted guidelines for the ethical use and care of laboratory animals.

Results

Result of clinical signs and morphological study:

Clinical observations

No observable clinical symptoms, behavioral changes, or external morphological abnormalities were detected in mice treated with aqueous extract of *J. phoenicea* (40 mg/kg/day) compared with the control group. However, dry feces containing dark granules were noted. In contrast, ethanol-treated mice exhibited reduced appetite, decreased body fur density, reduced activity, respiratory difficulty, and impaired movement with dizziness. These adverse clinical signs were markedly alleviated in mice treated with aqueous extract of *J. phoenicea* together with ethanol.

Mortality

No mortality was recorded in the control or extract-treated groups during the experimental period. Mortality reached 30% in the ethanol-treated group but decreased to 8% in mice receiving aqueous extract of *J. phoenicea* with ethanol.

Body weight

Ethanol administration caused a significant reduction in body weight gain, with final body weight decreasing by 15.1% relative to the initial weight and showing a significant decline compared with the control group. Mice treated with aqueous extract of *J. phoenicea* alone showed a 7.6% increase in final body weight relative to the initial weight, with no significant difference compared with the control group. The ethanol + extract group showed significant improvement in body weight, with a final increase of 10.5% relative to the initial weight (Table 1).

Result of haematological study

Hematological results

The ethanol-treated group showed a significant increase in hemoglobin (Hb), packed cell volume (PCV), and granulocyte percentage, accompanied by a significant decrease in lymphocyte percentage compared with the control group. Other hematological parameters, including RBCs, WBCs, platelet count, MCV, MCH, and MCHC, showed no significant changes.

The group treated with aqueous extract of *J. phoenicea* alone exhibited no significant alterations in most hematological parameters, except for a significant increase in Hb concentration.

Mice treated with ethanol followed by aqueous extract of *J. phoenicea* showed significant increases in Hb, RBCs, and PCV, together with marked improvement in lymphocyte and granulocyte percentages compared with the ethanol-treated group. In addition, WBC count showed a noticeable but statistically insignificant increase (Tables 2–4).

Light microscopic results

Hematoxylin and eosin-stained sections of the glandular stomach from the control group showed normal histological architecture (Fig. 1). Similarly, mice treated with aqueous extract of *J. phoenicea* alone exhibited normal gastric histology without detectable alterations (Fig. 2).

Table 1: Effect of Aqueous extract *of J. phoenicea* with or without ethanol on body weight gain of mice:

	Standards		
Groups	**Mean of Initial body weight (gm)**	**Mean of final body weight (gm)**	**Mean change in body weight gain (%)**
Control	21 ± 0.73^{a}	24 ± 0.81^{b}	14.2%
Ethanol	21 ± 0.8^{a}	18 ± 0.5^{c}	-15.1%
J. phoenicea	21 ± 0.1^{a}	22 ± 0.6^{a}	7.6%
Ethanol + *J. phoenicea*	21 ± 0.75^{a}	24 ± 1.1^{b}	10.5%

Data are presented as mean ± standard error (SE) of body weight for surviving animals in each experimental group. Means within the same row or column that do not share identical superscript letters are considered significantly different at $P \leq 0.05$.

Table 2: Effect of aqueous extract of *J. phoenicea* with or without ethanol in Peripheral Blood Picture

	Standards				
Groups	**Hb**	**RBCs**	**PCV%**	**WBC**	**Platelets**
Control	13.7 ± 0.00^{a}	9.26 ± 0.12^{a}	38.05 ± 0.25^{a}	4.64 ± 0.73^{a}	**427 ± 1.84^{a}**
Ethanol	15.5 ± 0.40^{b}	10.8 ± 0.21^{ab}	44.1 ± 1.24^{b}	5.33 ± 2.15^{a}	**610 ± 61.9^{a}**
J.Phoence	15.8 ± 0.17^{b}	10.8 ± 0.08^{ab}	42.03 ± 1.07^{ab}	9.01 ± 1.64^{a}	**605 ± 42.5^{a}**
Ethanol+ *J.Phoenice*	15.8 ± 0.57^{b}	11.7 ± 1.00^{b}	45.76 ± 2.35^{b}	16.0 ± 0.98^{b}	**665 ± 2.36^{a}**

Data are expressed as mean ± standard error (SE) based on five animals per group. Within each column, means that do not share the same superscript letter are considered statistically different at $P \leq 0.05$. Hematological parameters assessed included hemoglobin concentration (Hb), red blood cell count (RBCs), packed cell volume (PCV), total white blood cell count (WBCs), and platelet count.

Table 3: Effect of aqueous extract of *J. phoenicea* with or without ethanol in white blood cells.

	Standards		
Groups	**Lymphocytes, (%)**	**Monocytes, (%)**	**Granulocytes, (%)**
Control	69.55 ± 2.25^{a}	2.45 ± 0.77^{a}	27.95 ± 1.35^{a}
Ethanol	56.66 ± 2.49^{b}	3.10 ± 0.35^{a}	40.26 ± 2.75^{b}
J.Phoenice	70.43 ± 6.02^{a}	3.93 ± 0.58^{a}	25.6 ± 5.51^{a}
Ethanol+ *J.Phoenice*	70.16 ± 1.43^{a}	3.80 ± 0.55^{a}	26.06 ± 1.96^{a}

Data are presented as mean ± standard error (SE) calculated from five animals in each group. Means within the same column that do not share identical superscript letters differ significantly at $P \leq 0.05$. Differential leukocyte counts included lymphocytes, monocytes, and granulocytes

Table4: Effect Aqueous extract of *J. phoenicea* with or without ethanol in hematological parameters

Groups	Standars		
	MCV, (fL)	MCH, (Pg)	MCHC, (%)
Control	41.50± 0.28[a]	14.80± 0.23[a]	**35.95± 0.25[a]**
Ethanol	40.66± 0.88[a]	14.26± 0.13[a]	**35.13± 0.83[a]**
J. Phoenicea	38.66± 0.88[a]	14.50± 0.30[a]	**25.76± 10.58[a]**
Ethanol+*J. Phoenicea*	39.33± 1.20[a]	13.50± 0.65[a]	**34.50± 0.55[a]**

Results are expressed as mean ± standard error (SE) based on five animals per experimental group. Means within the same column that do not share identical superscript letters are considered statistically significant at $P \leq 0.05$. Evaluated erythrocyte indices included mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

In contrast, the ethanol-treated group showed marked pathological lesions, including erosion of the mucosal lining epithelium, reduced mucus covering, hemorrhage in the lamina propria with congested blood vessels, edema in the lamina propria and submucosa, inflammatory cell infiltration, connective tissue degeneration, and necrosis of many epithelial cells (Fig. 3).

Mice treated with aqueous extract of *J. phoenicea* following ethanol exposure demonstrated clear histological improvement, with restoration of mucosal integrity and reduction of inflammatory and degenerative changes (Fig. 4).

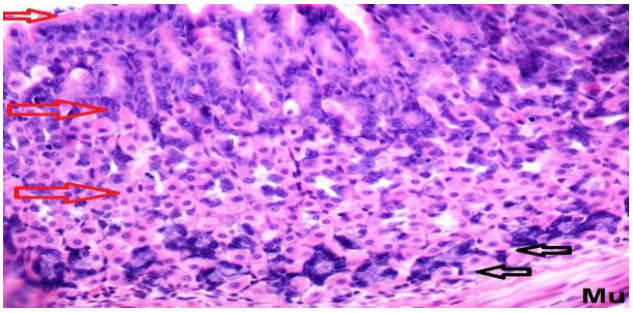

Figure 1: A section of the glandular stomach of a mouse from the control group showing tunica mucosa (Red Arrow), the natural regulation of connective tissue in tunica submucosa (Arrows), Tunica muscularis (Mu) (H&E stain, 200X).

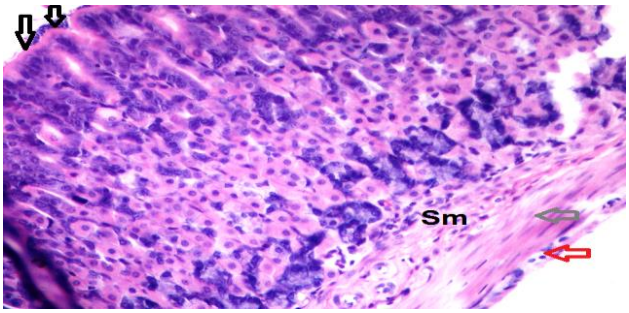

Figure 2: A section of glandular stomach of mouse treated with aqueous extract of J. *phoenicea* showing normal appearance of tunica mucosa (Arrows), Tunica submucosa (Sm), Tunica muscularis (Grey Arrow), Tunica serosa (Red Arrow) (H&E stain, 200X).

Altman's Stain Reactivity in Gastric Glandular Epithelium under *J. phoenicea* Extract and Ethanol Treatments: sections revealed increased reactivity of the mucous, secretory granules and mitochondria in some glandular epithelial cells with this stain in glandular stomach sections of mice treated orally with aqueous extract of *J. phoenicea* only compared to the control group in Figures 5 and 6. The ethanol group showed hemorrhage between gastric glands and congestion in the blood vessels. As well, the endothelial cells of the gastric glands showed a clear reduction of the positive interaction of Altman's stain with the mitochondria and the secretory granules in Figure 7. In contrast, an obvious decrease in the positive reactivity of mitochondria and secretory granules in gastric gland cells was noticed. An ameliorating in gastric glands feature and positive reactive of mitochondria and secretory granules in the glandular stomach tissue of mice co-treated with ethanol and aqueous extract of *J. phoenicea* was detected in Figure 8.

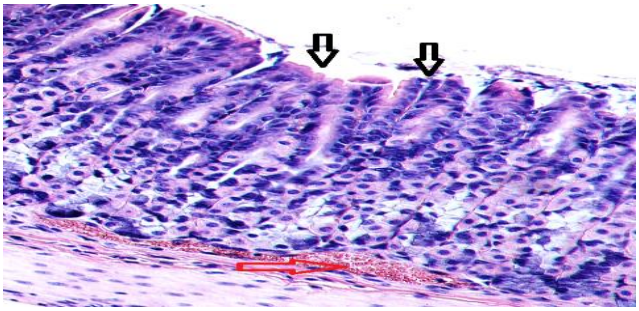

Figure 3: A section of the glandular stomach of a mouse treated with ethanol only showing erosion of epithelial cells lined tunica mucosa with (Arrows), haemorrhage in lamina propria with congested blood vessel (Red Arrow) (H&E stain, 200X).

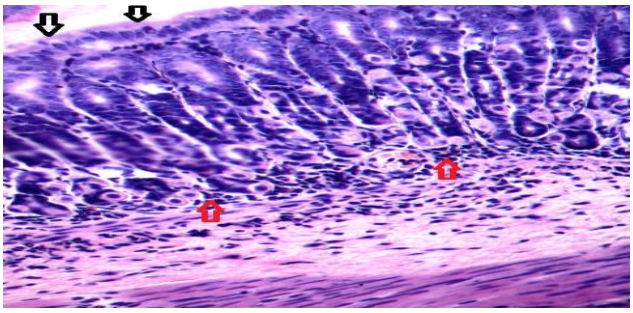

Figure 4: A section of the glandular stomach of a mouse Co- treated with ethanol and aqueous extract of *J. phoenicea* showing an improvement in tunica mucosa lining epithelium (Arrows), Inflammatory cells infiltration (Red Arrows) (H&E stain, 200X).

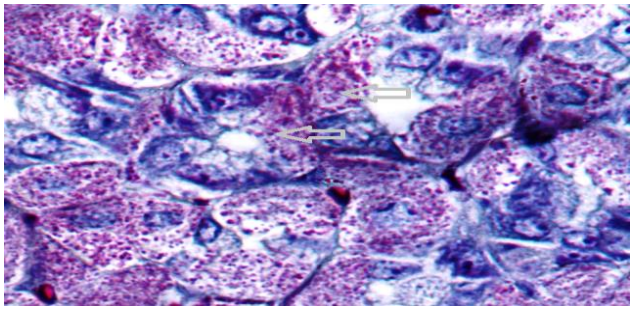

Figure 5: A section of glandular stomach of mouse from the control group showing normal positive reactivity of mitochondria and secretory granules in gastric glands (Grey Arrows) (Altman's technique-stained sections, 1000X).

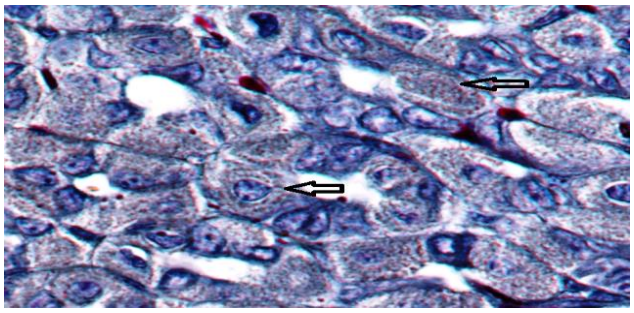

Figure 6: A section of glandular stomach of mouse treated with aqueous extract of *J. phoenicea* showing normal appearance of gastric glands (Arrows) with positive reactive of mitochondria and secretory granules in (Altman's technique stained sections, 1000X).

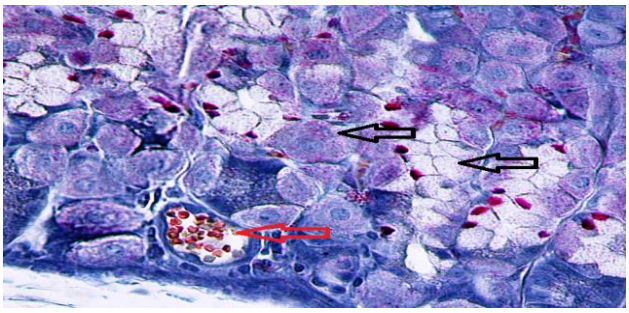

Figure 7: A section of the glandular stomach of a mouse treated with ethanol only showing an obvious decrease in the positive reaction of mitochondria and secretory granules in gastric gland cells (The Arrows), Congested blood vessel (Red Arrows) (Altman's technique stained sections, 1000X).

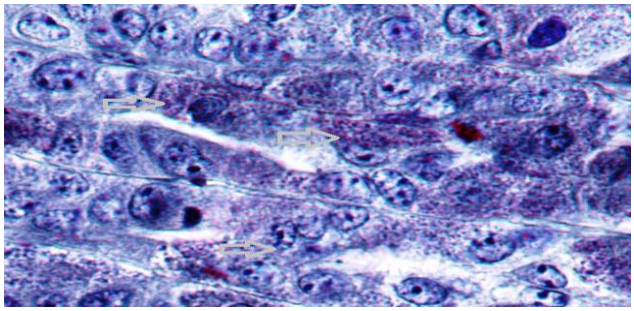

Figure 8: A section of glandular stomach of mouse Co- treated with ethanol and aqueous extract of *J. phoenicea* showing amelioration in gastric glands feature and positive reactive of mitochondria and secretory granules (Grey Arrows) (Altman's technique stained sections, 1000X).

Transmission electron microscopy (TEM) results:

Ultrastructural examination of the gastric mucosa in **the control group** revealed normal simple columnar epithelial cells with intact apical microvilli, oval nuclei containing heterochromatin, and abundant apical secretory granules. The cytoplasm contained normal mitochondria, ribosomes, rough endoplasmic reticulum, and Golgi apparatus (Fig. 9).

In the ethanol-treated group, epithelial cells showed marked surface fragmentation, depletion of secretory granules, reduced mitochondria, swelling of rough endoplasmic reticulum, and decreased ribosomes. Vascular congestion in the lamina propria was also observed (Fig. 10).

Mice treated with aqueous extract of ***J. phoenicea*** **alone** exhibited generally normal epithelial ultrastructure, with preserved nuclei, mitochondria, ribosomes, and Golgi apparatus, although a slight reduction in secretory granules was noted (Fig. 11).

The ethanol + extract group demonstrated clear ultrastructural improvement, including restoration of the apical epithelial surface, normal nuclear chromatin distribution, and recovery of rough endoplasmic reticulum compared with the ethanol-treated group (Fig. 12).

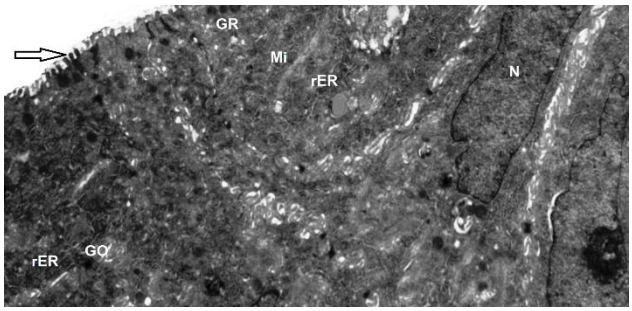

Figure 9: Picture with Transmission electron microscope of the mucosal laer of the stomach of mice in the control group, epithelial cells (Arrows),oval nucleus (N),Golgi apparatus (GO), mitochondria (Mi), rough endoplasmic reticulum (rER), secretory granules (GR), (Uranyl acetate and lead citrate, 27500X).

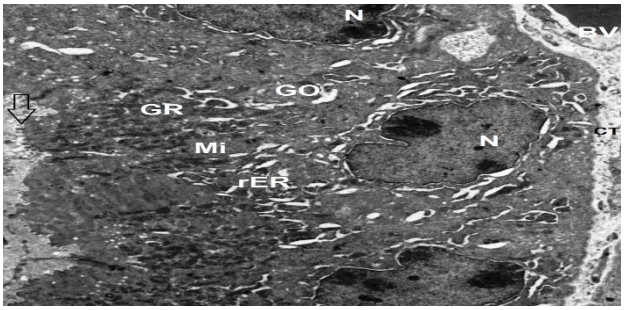

Figure 10: Picture with Transmission electron microscope of the mucosal layer of the stomach mice in the ethanol group, fragmentation of the epithelial cells surface (Arrows), oval nucleus (N), Golgi apparatus (GO), mitochondria (Mi), rough endoplasmic reticulum (rER), blood vessel (BV), secretory granules (GR), (Uranyl acetate and lead citrate , 20600X).

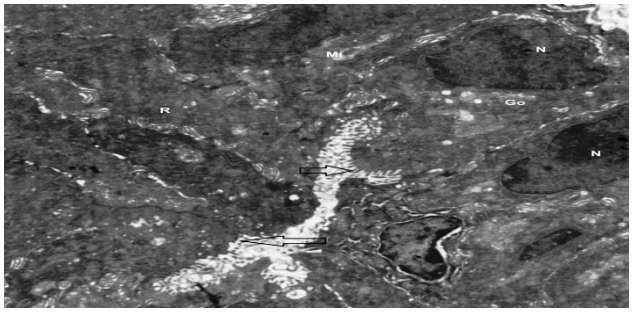

Figure 11: Picture with Transmission electron microscope in aqueous extract of *j. phoenicea* group , epithelial cells (Arrows), oval nucleus (N), Golgi apparatus (GO), mitochondria (Mi), ribosomes (R) (Uranyl acetate and lead citrate , 27500X).

Discussion

Histological examination of the stomach of ethanol-treated mice revealed marked mucosal erosion, destruction of gastric epithelial cells, hemorrhage, vascular congestion, edema, and inflammatory cell infiltration. Transmission electron microscopy (TEM) further confirmed epithelial surface disruption and depletion of cellular organelles [22]. Ethanol is known to penetrate the gastric mucosa and induce direct

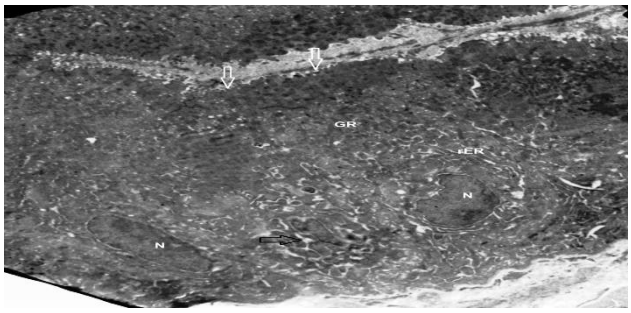

Figure 12: Picture with Transmission electron microscope in Ethanol + *J. phoenicea* treatment group, epithelial cells (White Arrows),oval nucleus (N), secretory granules (GR), rough endoplasmic reticulum (rER), fragmentation to some epithelial cells (Arrow), (Uranyl acetate and lead citrate , 27500X).

cellular injury, leading to epithelial exfoliation and ulcer formation. This damage is largely mediated by excessive generation of reactive oxygen species (ROS), which promote lipid peroxidation and oxidative injury to cellular membranes and tissue components [23,24]. In addition, ethanol weakens the gastric mucosal barrier, reducing its resistance to acid and digestive enzymes, thereby aggravating erosion and necrosis. These findings are consistent with previous reports [25].

The widespread use of medicinal plants in traditional therapy has raised important concerns regarding their long-term safety and potential biological effects [32]. In the present study, gastric tissues from mice treated with aqueous extract of *J.phoenicea* alone exhibited no detectable histopathological alterations, indicating a favorable safety profile under the experimental conditions. Moreover, the marked improvement observed in ethanol-intoxicated mice following treatment with the extract may be attributed to its potent antioxidant and anti-inflammatory properties. These findings are consistent with previous reports demonstrating that *J. phoenicea* enhances endogenous antioxidant defense systems and mitigates oxidative stress by inhibiting lipid peroxidation while upregulating key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [9]. Collectively, these results suggest that despite general concerns about the prolonged use of herbal medicines, *J. phoenicea* exhibits a protective and relatively safe profile, particularly in the context of oxidative stress-induced gastric damage. Preservation of mitochondrial integrity and epithelial cell membranes may explain the recovery of mucosal structure observed in the present study.

The anti-inflammatory effect of the extract may also contribute to tissue healing. Ethanol exposure activates macrophage polarization and stimulates pro-inflammatory cytokines such as TNF-α and IL-17 through NF-kappaB and PI3K-Akt signaling pathways [25]. Bioactive constituents of *J. phoenicea*, particularly flavonoids and terpenoids, may modulate these pathways, reducing neutrophil infiltration and supporting epithelial regeneration. These mechanisms may explain the marked reduction in vascular congestion, hemorrhage, and inflammatory lesions in the treated group.

Ultrastructural findings further supported this protective effect. TEM examination of the ethanol + extract group showed restoration of epithelial surface integrity, improved rough endoplasmic reticulum, normal chromatin distribution, and recovery of secretory granules compared with ethanol-treated animals. Previous studies have also reported that juniper preparations improve digestive function, stimulate secretion of digestive fluids, relieve gastrointestinal discomfort, and possess wound-healing properties [26,27]. These effects may be partly related to enhancement of haemostasis and epithelial proliferation, thereby accelerating tissue repair [10].

Regarding morphological findings, ethanol-treated mice showed reduced appetite, decreased activity, respiratory distress, dizziness, and body weight loss, whereas these abnormalities were markedly alleviated after treatment with *J. phoenicea* extract. Improvement in feeding behavior and body weight may reflect recovery of gastric function and reduced mucosal injury.

Hematological results also supported the therapeutic role of the extract. Ethanol exposure caused significant increases in Hb, RBCs, and PCV, possibly due to compensatory erythropoietic responses mediated by erythropoietin stimulation [28], together with leukocyte alterations characterized by increased granulocytes and decreased lymphocytes, indicating inflammatory stress. Administration of *J. phoenicea* significantly improved these parameters and partially restored leukocyte balance, suggesting antioxidant and immunomodulatory effects [29], [30]. Platelet counts showed mild non-significant increases, which may be associated with thrombopoietin activity and inflammatory responses [31].

Recent studies have further supported the therapeutic relevance of *J. phoenicea* in ethanol-induced gastric injury through its ability to reduce oxidative stress and inflammatory responses. Previous reports indicated that the extract may lower lipid peroxidation markers such as MDA while enhancing antioxidant enzyme activity, including SOD, CAT, and GPx. These biochemical effects were associated with preservation of gastric mucosal integrity, reduction of inflammatory infiltration, and enhanced epithelial regeneration [2]. In addition, phytochemical screening identified flavonoids, terpenoids, and phenolic compounds that may synergistically stabilize gastric epithelial membranes and suppress oxidative injury. Such findings are in close agreement with the present results and support the potential use of *J. phoenicea* as a complementary agent in gastric ulcer management [2].

Conclusion

The findings of this study indicate that *J. phoenicea* extract possesses notable preventive and therapeutic potential against ethanol-induced gastric injury when administered at scientifically validated and controlled doses. Its bioactive constituents may contribute to mucosal protection, antioxidant defense, and tissue regeneration. However, the use of medicinal plants should always be guided by evidence-based protocols and performed under professional medical supervision, given the possibility of herb drug interactions when co-administered with conventional medications. Further clinical and mechanistic studies are warranted to confirm its safety profile, elucidate its molecular targets, and explore its integration into complementary gastroprotective therapies.

Recommendations

- Advanced phytochemical analysis is recommended to identify the active constituents responsible for the therapeutic effects of *J. phoenicea* extract.
- Additional molecular studies are needed to clarify the mechanisms underlying its gastroprotective activity.
- Comparative studies with standard anti-ulcer drugs are recommended to determine its therapeutic value.

Author Contributions: All authors contributed equally to the conceptual framework and overall design of the study.

Experimental implementation, animal handling and treatment protocols, data acquisition, and statistical evaluation were conducted collaboratively. Histological and ultrastructural analyses, including light microscopy and transmission electron microscopy, were jointly performed, followed by collective data interpretation and figure preparation. The manuscript was written through shared efforts and subsequently revised critically by all authors. Final approval of the submitted version was granted by every author.

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