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Gastroprotective Potential of Wild-Grown *Rheum Rhabarbarum* From Northern Iraq: A Preliminary Pharmacological Study on Ethanol-Induced Gastric Ulcers in Rat

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Abstract

Introduction: *Rheum rhabarbarum* (Rhubarb), locally known as "Rivas," is a traditional medicinal plant widely consumed in Northern Iraq for various gastrointestinal ailments. Although it has an extensive ethnomedical history, the specific gastroprotective efficacy of the local Iraqi ecotype remains scientifically under-documented.

Methods: Twenty-four Sprague Dawley rats were assigned to four groups: normal control, *R. rhabarbarum* methanolic extract (500 mg/kg), ulcer control (96% ethanol) and positive control (Omeprazole 20 mg/kg). Mucus weight, gastric pH and ulcer inhibition rates were calculated. Oxidative stress markers (CAT, SOD, MDA) and inflammatory cytokines (IL-10, IL-6, TNF-α) were analyzed in gastric tissues.

Results: The Iraqi *R. rhabarbarum* extract demonstrated a significant ulcer inhibition rate of 82.0%, which was comparable to the Omeprazole group (84.0%). The extract significantly reduced pro-inflammatory cytokines (TNF-α, IL-6) and MDA levels, while significantly elevating antioxidant enzyme activities (CAT, SOD) and IL-10 levels ($p < 0.05$).

Conclusion: This preliminary study provides the first pharmacological evidence supporting the traditional use of Iraqi *R. rhabarbarum as* a potent gastroprotective agent. Its efficacy is mediated through a synergistic mechanism of antioxidant defense, mucosal protection and anti-inflammatory modulation.

الإمكانات الوقائية المعدية لنبات الراوند البري النامي في شمال العراق: دراسة دوائية أولية على القرح المعدية المستحدثة بالإيثانول في الجرذان

هالو حميد، أشرف جليك، يعقوب جم يابابا، غامزة موجان، حواء جوبان أوغلاري

الملخص

المقدمة: يُعد **Rheum rhabarbaru**(الراوند)، المعروف محليًا باسم **ريفاس**، من النباتات الطبية التقليدية الشائعة الاستخدام في شمال العراق لعلاج العديد من اضطرابات الجهاز الهضمي. وعلى الرغم من تاريخه الطويل في الطب الشعبي، فإن فعاليته الوقائية على مخاطية المعدة، ولا سيما للنمط البيئي العراقي، لا تزال بحاجة إلى توثيق علمي.

الهدف: هدفت هذه الدراسة إلى تقييم التأثير الوقائي للمستخلص الميثانولي لنبات **Rheum rhabarbarum** العراقي ضد القرح المعدية المستحدثة بالإيثانول في الجرذان، مع استقصاء دوره في تعديل مؤشرات الإجهاد التأكسدي والالتهاب.

المواد والطرائق: أُجريت الدراسة على 24جرذًا من نوعSprague–Dawley ، ووزعت عشوائيًا إلى أربع مجموعات: مجموعة السيطرة الطبيعية، ومجموعة القرحة المستحثة بالإيثانول (96%)، ومجموعة السيطرة الإيجابية المعالجة بالأوميبرازول بجرعة 20 ملغم/كغم، ومجموعة المعالجة بالمستخلص الميثانولي لنبات R. rhabarbarumبجرعة 500ملغم/كغم .وشملت التقييمات قياس الرقم الهيدروجيني لعصارة المعدة، ووزن المخاط المعدي، ونسبة تثبيط القرحة، إضافةً إلى تقدير مؤشرات الإجهاد التأكسدي، بما في ذلك المالون ثنائي الألدهيد(MDA) ، وإنزيمي Superoxide Dismutase (SOD)وCatalase (CAT)، فضلًا عن قياس مستويات السيتوكينات الالتهابية IL-6وTNF-αوالسيتوكين المضاد للالتهاب IL-10في أنسجة المعدة.

النتائج: أظهر المستخلص الميثانولي لنبات **R. rhabarbaru**العراقي تأثيرًا وقائيًا ملحوظًا ضد القرح المعدية، إذ بلغت نسبة تثبيط القرحة **82.0%**، وهي نسبة مقاربة لما تحقق في مجموعة الأوميبرازول .(**84.0%**) كما أدى العلاج بالمستخلص إلى انخفاض معنوي في مستويات **MDA**و **IL-6**و**TNF-α**، مع زيادة معنوية في نشاط الإنزيمين المضادين للأكسدة **SOD**و**CAT**، بالإضافة إلى ارتفاع مستوى **IL-10**مقارنةً بمجموعة القرحة غير المعالجة.(**P < 0.05**)

الاستنتاج:تشير نتائج هذه الدراسة الأولية إلى أن المستخلص الميثانولي لنبات Rheum rhabarbarumالعراقي يمتلك تأثيرًا وقائيًا واعدًا ضد الأذية المخاطية المعدية المستحثة بالإيثانول. ويُرجح أن تعزى هذه الفعالية إلى التأثيرات المضادة للأكسدة والمضادة للالتهاب، فضلًا عن تعزيز آليات الدفاع المخاطية للمعدة. وتدعم هذه النتائج الاستخدام التقليدي للنبات، كما تمهد لإجراء دراسات دوائية وآلياتية وسريرية مستقبلية لتأكيد فعاليته وسلامته.

1. Introduction

Gastric ulcer remains a clinically relevant disorder that arises when oxidative injury and aggressive luminal factors overwhelm mucosal defense systems such as prostaglandins, mucus, bicarbonate, epithelial restitution and microcirculation(Lanas and Chan, 2017; Vakil, 2024). Although proton pump inhibitors (PPIs) remain a cornerstone of therapy, unnecessary or prolonged use has raised concern because of associations with fractures, nutrient deficiencies, gastrointestinal infections and kidney disease reinforcing interest in safer alternative or adjunctive gastroprotective agents derived from medicinal plants (Freedberg, Kim and Yang, 2017; Vakil, 2024)

Within this context, the genus Rheum (Polygonaceae) is of particular pharmacological interest. Rheum species have long been used in traditional medical systems for inflammatory, digestive and hepatobiliary disorders, and they contain a broad range of bioactive metabolites including anthrones, anthraquinones, flavonoids, stilbenes, chromones and other polyphenolic compounds (Cao *et al.*, 2017; Xiang *et al.*, 2020).These constituents have been linked to , anti-inflammatory, antioxidant, cytoprotective, antimicrobial effects, all of which are mechanistically relevant to gastric mucosal protection (Cao *et al.*, 2017; Xiang *et al.*, 2020).

In Northern Iraq and the wider Kurdistan region, wild medicinal and edible plants continue to play a crucial role in community health practices. Ethnobotanical work from Sulaymaniyah and neighboring Kurdish areas has shown the persistence of plant-based remedies for inflammatory and gastrointestinal complaints, supporting the scientific value of investigating locally used taxa (Ahmed, 2016). Based on its traditional use in the region, edible Rheum taxa are usually referred to as “Rivas,” and their aerial roots and parts are traditionally consumed for digestive complaints. However, despite this strong ethnomedical background, the gastroprotective activity of the local Iraqi Rheum ecotype has not been adequately characterized under controlled experimental conditions (Ahmed, 2016).

The ethanol-induced gastric ulcer model is particularly suitable for this purpose because it reproduces rapid hemorrhagic injury driven by mucus depletion, oxidative stress, inflammatory cytokine release, microvascular disturbance and apoptotic signaling(Zorofchian *et al.*, 2014; Lanas and Chan, 2017; Raish *et al.*, 2021). Therefore, the present study was designed to evaluate the gastroprotective potential of wild-grown Iraqi Rheum rhabarbarum using macroscopic, histopathological, biochemical and immunohistochemical endpoints. By linking regional traditional knowledge with experimentally measurable mechanisms, this study aims to provide evidence that may support the standardization and future development of an Iraqi plant-derived gastroprotective candidate(Cao et al., 2017;Ahmed, 2016)

2. Materials and Methods

2.1. Plant Material and Extraction Process

The roots of wild-grown *Rheum rhabarbarum* were harvested during the spring season from the mountainous terrains of Northern Iraq. The plant material was identified based on its morphological characteristics and available botanical references, in accordance with previous studies on Rheum species from Iraq. No formal voucher specimen was assigned for the collected material. The collected roots were thoroughly washed with distilled water, air-dried in the shade at room temperature and consequently ground into a fine powder using an electric blender.

For the extraction, 100g of the powdered root was macerated in 500 mL of 90% methanol at room temperature for 72 hours with occasional shaking. The mixture was filtered through Whatman No. 1 filter paper. The filtrate was then

concentrated under reduced pressure using a rotary evaporator at 40°C to obtain a crude methanolic extract. The final extract was stored in airtight sterile amber vials at -20°C until further pharmacological use.

2.2.Animals and Ethical Approval

Healthy adult Sprague-Dawley rats (weighing 200–250 g) of both sexes were obtained from the Experimental Animal Research Center. The animals were housed in clean polycarbonate cages under standard laboratory conditions (22 ± 2°C, 12-hour light/dark cycle) and provided with a water ad libitum and standard pellet diet. All experimental protocols were reviewed and approved by the Animal Ethics Committee of Cihan University in accordance with the Guide for the Care and Use of Laboratory Animals.

2.3.Experimental Design and Ulcer Induction

The rats were randomly assigned to four experimental groups (n=6 per group):

1. Normal Control: Received only the vehicle (1% Tween 20).
2. Ulcer Control: Received 96% ethanol (1 mL/200g) via oral gavage to induce gastric injury.
3. Positive Control: Pre-treated with Omeprazole (20 mg/kg, p.o.) 60 minutes before ethanol administration.
4. Extract Group: Pre-treated with *R. rhabarbarum* methanolic extract (500 mg/kg, p.o.) 60 minutes before ethanol administration.

All rats were fasted for 24 hours prior to the experiment but had free access to water. One hour after the ethanol challenge, the animals were euthanized under deep anesthesia.

2.4.Macroscopic Evaluation and Mucus Measurement

The stomachs were removed, opened along the greater curvature and rinsed with ice-cold saline. The gastric mucosal lesions were examined at macroscopic level. The ulcer area (mm^2) was measured and the Ulcer Index (UI) and Percentage of Inhibition (%) were calculated using standard formulas. Alcian Blue binding method was used to determine Gastric wall mucus as previously described.

2.5.Biochemical Analysis of Gastric Tissue

A portion of the gastric tissue was homogenized in ice-cold phosphate-buffered saline (pH 7.4, PBS). The homogenates were centrifuged at 4000 rpm for 15 minutes at 4°C. The supernatants were collected to determine: Inflammatory Cytokines Levels of Tumor Necrosis Factor-alpha (TNF-α) and Interleukin-6 (IL-6) were quantified using specific rat ELISA kits according to the manufacturer's instructions.

Oxidative Stress Markers: Malondialdehyde (MDA) levels (as an index of lipid peroxidation), Superoxide Dismutase (SOD) and Catalase (CAT) activities using commercially available colorimeter, ic kits.

2.6.Histopathological and Immunohistochemical Studies

The remaining gastric tissues were fixed in 10% buffered formalin for 24 hours. After routine processing and paraffin embedding, 5-µm thick sections were cut and stained with Periodic Acid-Schiff (PAS) for mucus identification and Hematoxylin and Eosin (H&E) for general morphology. For immunohistochemistry, sections were incubated with primary antibodies against HSP-70 (cytoprotective marker), Bax (pro-apoptotic marker). The expression levels were evaluated by a pathologist blinded to the treatment groups.

2.7.Statistical Analysis

All experimental data were presented as mean ± standard error of the mean (SEM). GraphPad Prism software (version 8.0) was used to perform statistical analysis. The normality of data distribution was assessed using the Shapiro-Wilk test. Differences between the experimental groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons to determine specific group differences. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant. Graphical representations were generated to illustrate the comparative efficacy of the treatment groups.

3. Results

3.1.Acute Toxicity and Safety Profile

The acute toxicity study showed that oral administration of *R. rhabarbarum* extract at single doses of 2.5 g/kg and 5.0 g/kg did not result in observable clinical signs of toxicity over the 14-day monitoring period or any mortality. Furthermore, biochemical assessments of renal (potassium, sodium, urea, chloride and creatinine) and hepatic (ALT, AST) parameters remained within normal physiological ranges (Supplementary Tables S1–S4). These results indicate that the methanolic extract of the local Iraqi ecotype is non-toxic at the tested pharmacological doses.

3.2.Evaluation of Macroscopic Gastric Lesions

The administration of 96% ethanol induced severe gastric mucosal damage, characterized by deep erosions and extensive hemorrhagic streaks in the ulcer control group (Table1). In contrast, pre-treatment with both the R. rhabarbarum extract and Omeprazole significantly reduced the severity of these lesions. Quantitative analysis showed a significant decrease in the total ulcer area in all treated groups compared to the ulcer control ($p < 0.05$). Importantly, the 500 mg/kg dose of the extract achieved an inhibition rate of 82.0%, which was highly comparable to the 84.0% inhibition observed in the Omeprazole-treated positive control group (Fig.1A-F).

Table1: Gastroprotective Effects of *R. Rhabarbarum* Extract on Ethanol-Induced Gastric Ulcer Parameters.

Group	Pre-treatment	Dose	Ulcer Area (mm^2)	pH	Mucous Weight (g)	Ulcer Inhibition (%)
1	Normal Control	0.5 mL	0.0±0.0	5.32±0.84	0.59±0.15	0.00
2	Ulcer Control	-	911.83±33.51	0.13±0.36	0.13±0.36	0.00
3	Omeprazole	20 mg/kg	145.13±21.33	0.18±1.57	0.18±1.57	84%
4	Extract (Low)	250 mg/kg	186.17±22.26	0.13±1.11	0.13±1.11	79.5%
5	Extract (High)	500 mg/kg	163.51±24.20	0.11±1.33	0.11±1.33	82%

All values are expressed as Mean ± S.E.M. (n=6 per group). One-way ANOVA followed by Tukey's post-hoc test was used for statistical analysis. Superscript letters indicate significant differences at $p < 0.05$: (a) when compared to the Normal Control group; (b) when compared to the Ulcer Control group. *R. rhabarbarum: Rheum rhabarbarum.*

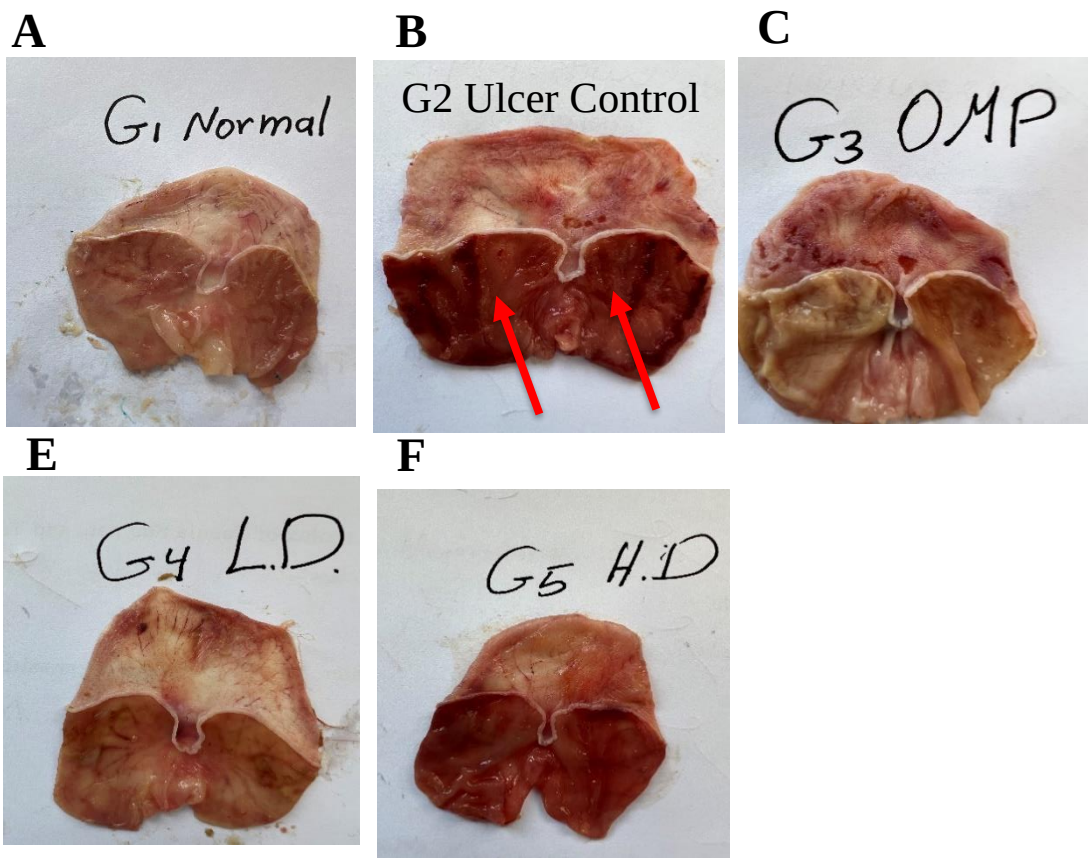

Figure1: Macroscopic Appearance of The Gastric Mucosa In The Ethanol-Induced Ulcer Model. Representative images of the stomach linings from different experimental groups: A. Normal Control Group: Displays a healthy, intact gastric epithelium with no signs of injury. B. Ulcer Control Group (Ethanol 96%): Shows extensive and severe hemorrhagic lesions, deep longitudinal streaks, and significant mucosal congestion. C. Positive Control (Omeprazole 20 mg/kg): Exhibits a markedly preserved mucosal architecture with only minor, superficial damage. D & E. Rheum rhabarbarum Treated Groups (250 and 500 mg/kg): Demonstrate a significant, dose-dependent reduction in the area and severity of hemorrhagic ulceration, confirming the potent gastroprotective efficacy of the Iraqi plant extract against ethanol-induced acute injury

3.3.Effects on Intragastric pH and Mucus Content

Ethanol challenge significantly compromised the gastric defensive barrier, as evidenced by a a decrease in mucus weight and reduction in intragastric pH in the ulcer control group. Pre-treatment with *R. rhabarbarum* extract significantly countered these effects by increasing the gastric wall mucus weight and elevating the pH ($p < 0.05$). The high-dose extract (500 mg/kg) produced protective effects similar to those of Omeprazole, as detailed in Table2.

Table2: Impact of *R. Rhabarbarum* Extract on SOD, CAT, And MDA Levels In Gastric Tissue

Group	Pre-treatment	SOD (U/mg protein)	CAT (nmol/min/ml protein)	MDA (umol/L)
G1	Negative Control (0.5% CMC)	0.57	117	2.5
G2	Ulcer Control (0.5% CMC)	0.26	60	16
G3	Omeprazole (20 mg/kg)	1.04	135	3.5
G4	*R. rhabarbarum* (250 mg/kg)	0.75	109	5.5
G5	*R. rhabarbarum* (500 mg/kg)	0.91	125	4.03

Values represent the mean ± S.E.M. Administration of ethanol significantly reduced SOD and CAT activities and increased MDA levels ($p < 0.05$ vs. Normal Control). Pre-treatment with R. rhabarbarum extract (250 and 500 mg/kg) restored antioxidant enzyme activities and significantly reduced lipid peroxidation (MDA), demonstrating a dose-dependent protective effect comparable to omeprazole (20 mg/kg).

3.4. Histopathological Observations

Hematoxylin and Eosin (H&E) staining of the ulcer control group revealed significant pathological alterations, including submucosal edema, extensive epithelial loss and dense inflammatory cell infiltration. However, rats pre-treated with the extract showed minimal sub-mucosal edema and maintained epithelial continuity.

Periodic Acid-Schiff (PAS) staining further validated these findings; while the extract-treated groups demonstrated intense PAS positivity, the ulcer control group exhibited severe depletion of mucus glycoproteins (indicated by weak magenta coloration). This indicates that the Iraqi *R. rhabarbarum* extract enhances or preserves the production of protective gastric mucus (Fig.2, Fig.3 and Fig.4).

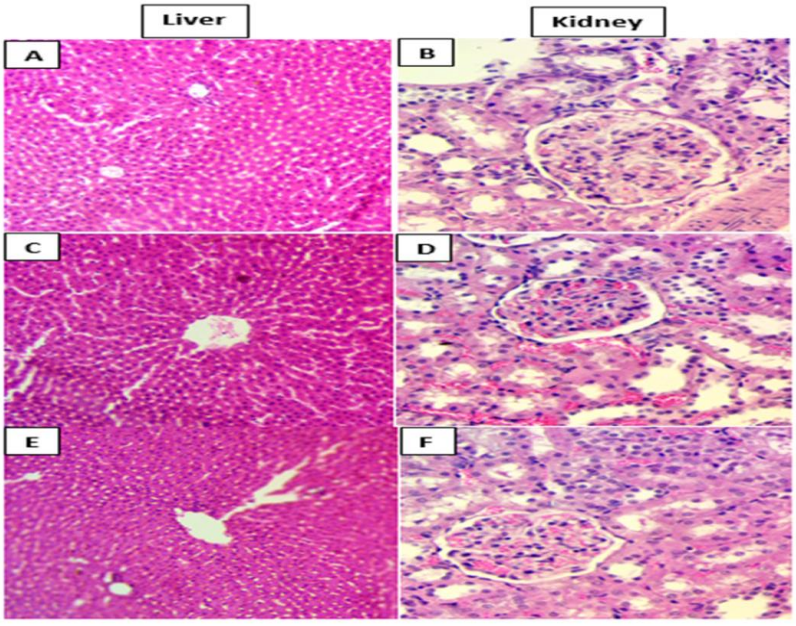

Figure2: Histopathological assessment of liver and kidney tissues in the acute toxicity study. Representative photomicrographs of tissue sections from rats treated with the vehicle (1% Tween 20, 5 mL/kg) are shown in A (Liver) and B (Kidney). Sections from rats treated with a 2500 mg/kg (2.5 g/kg) dose of R. rhabarbarum extract are represented in C (Liver) and D (Kidney), while sections from the 5000 mg/kg (5 g/kg) dose group are shown in E (Liver) and F (Kidney). No observable pathological alterations, structural deformities, or inflammatory foci were detected in the liver or kidney architectures of the extract-treated groups compared to the vehicle control. (Hematoxylin and Eosin staining, 20× magnification).

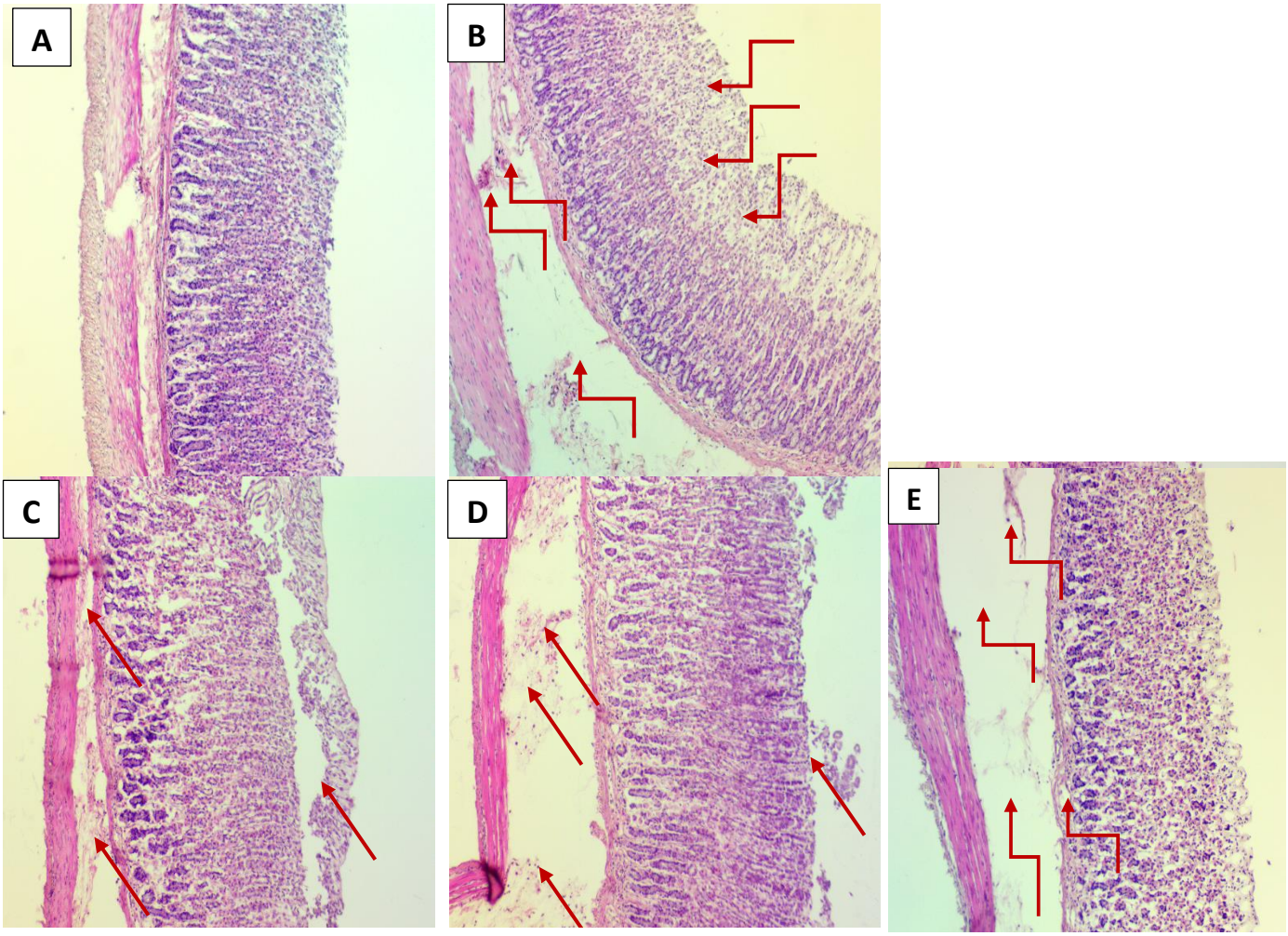

Figure3: Histopathological evaluation of the protective effects of Rheum rhabarbarum on ethanol-induced gastric mucosal injury (H&E staining). Representative photomicrographs of the gastric sections: (A) Normal Control Group: Shows a well-preserved histological architecture of the gastric mucosa with intact epithelium and glands. (B) Ulcer Control Group (Ethanol 96%): Exhibits profound structural damage, characterized by extensive epithelial exfoliation, prominent submucosal edema, and dense inflammatory cell infiltration (leukocyte recruitment indicated by red arrows). (C) Positive Control (Omeprazole 20 mg/kg): Displays significant restoration of the mucosal lining with only mild superficial desquamation. (D) R. rhabarbarum (250 mg/kg): Shows moderate protection with a visible reduction in necrotic areas compared to the ulcer control. (E) R. rhabarbarum (500 mg/kg): Demonstrates substantial gastroprotection with nearly intact epithelial continuity and minimal submucosal edema, confirming a dose-dependent efficacy of the extract. (Hematoxylin and Eosin stain, 10× magnification).

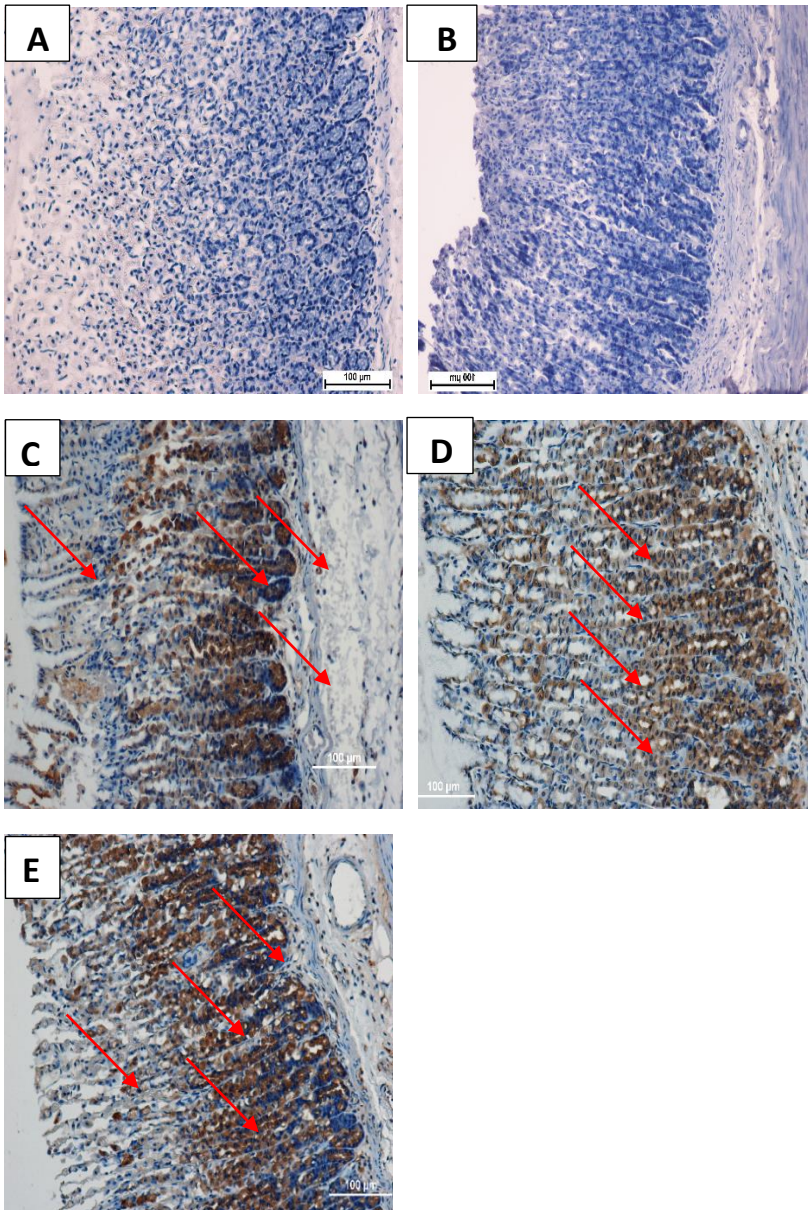

Figure4: Immunohistochemical Evaluation of Heat Shock Protein 70 (HSP-70) Expression in Gastric Tissues. Representative photomicrographs illustrating the cytoprotective response across different groups: (A) Normal Control Group: Shows a well-preserved gastric mucosa with baseline (minimal) HSP-70 immunoreactivity. (B) Ulcer Control Group: Exhibits extensive mucosal architecture disruption accompanied by a marked downregulation of HSP-70 expression, indicating a failed cellular defense against ethanol-induced stress. (C) Positive Control (Omeprazole 20 mg/kg): Displays noticeable upregulation of HSP-70 (identified by brown cytoplasmic staining) along with significantly preserved mucosal integrity. (D) R. rhabarbarum (250 mg/kg): Shows a moderate increase in HSP-70 expression compared to the ulcer control, correlating with reduced mucosal damage. (E) R. rhabarbarum (500 mg/kg): Demonstrates intense HSP-70 immunoreactivity and robust protection of the gastric glands, suggesting that the extract's gastroprotective effect is mediated by the induction of this cytoprotective chaperone protein. (HSP-70 staining, 20× magnification).

3.4. Immunohistochemical Findings: HSP-70 and Bax Expression

Immunohistochemical analysis showed that ethanol administration led to upregulation of the pro-apoptotic Bax protein and a downregulation of the cytoprotective Heat Shock Protein 70 (HSP-70) in the gastric tissue. Pre-treatment with *R. rhabarbarum* significantly reversed these markers. Specifically, the 500 mg/kg dose induced a strong brown-stained antigen expression for HSP-70, indicating high cytoprotective activity (Fig.5). Conversely, Bax protein expression, which was significantly elevated in the ulcerated control was significantly reduced in the extract-treated groups in a dose-dependent manner.

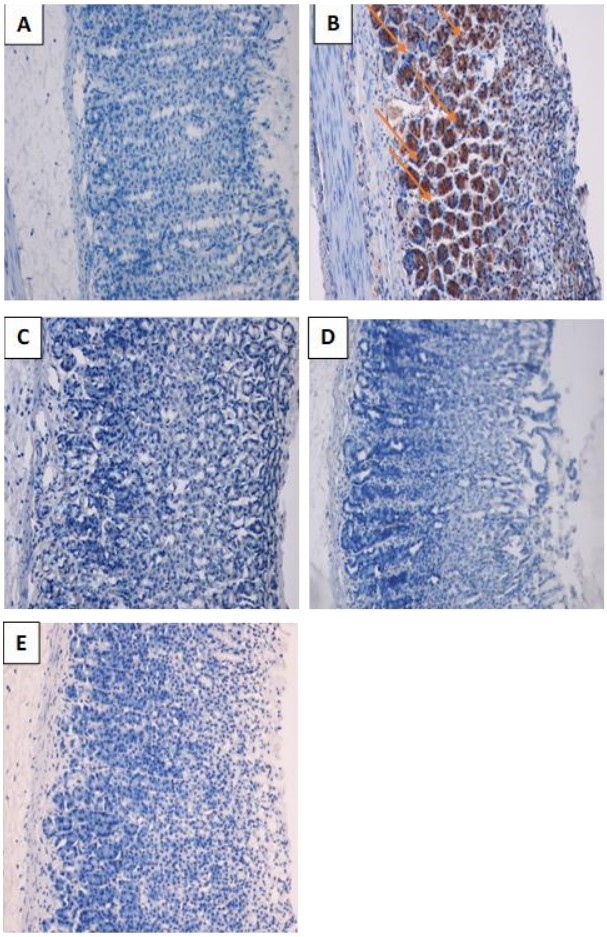

Figure5: Immunohistochemical Analysis of Pro-Apoptotic Bax Protein Expression in Gastric Mucosal Tissues. Representative photomicrographs demonstrating the apoptotic response across experimental groups: (A) Normal Control Group: Shows a healthy gastric architecture with minimal (baseline) Bax immunoreactivity. (B) Ulcer Control Group: Exhibits a marked increase in Bax protein expression (identified by intense brown staining, indicated by arrows), correlating with severe mucosal damage and increased programmed cell death. (C) Positive Control (Omeprazole 20 mg/kg): Displays significantly reduced Bax expression and preserved mucosal integrity compared to the ulcer control. (D) R. rhabarbarum (250 mg/kg): Shows a moderate decrease in Bax-positive cells, indicating a partial reduction in apoptotic signaling. (E) R. rhabarbarum (500 mg/kg): Demonstrates a substantial downregulation of Bax protein expression, suggesting that the extract's gastroprotective mechanism involves the inhibition of apoptosis and preservation of cellular viability. (Bax staining, 20× magnification)

3.5.Antioxidant Enzyme Activities and Lipid Peroxidation

Ethanol-induced oxidative stress was characterized by a significant elevation in MDA concentrations and a drastic reduction in CAT and SOD activities. Pre-treatment with the *R. rhabarbarum* extract effectively restored CAT and SOD activities while significantly lowering MDA levels ($p < 0.05$). The 500 mg/kg dose demonstrated a robust antioxidant effect, significantly mitigating lipid peroxidation in the gastric mucosa, as shown in Table2.

3.6.Modulation of the Cytokine Profile

The inflammatory response to ethanol-induced ulceration involved a significant increase in pro-inflammatory cytokines (IL-6 and TNF-α) and a decrease in the anti-inflammatory cytokine IL-10. All treatment groups showed a significant reduction in IL-6 and TNF-α levels while concurrently increasing IL-10 concentrations compared to the ulcer control ($p < 0.05$). The anti-inflammatory potency of the high-dose extract was comparable to that of Omeprazole as presented in Table3.

Table3: Effect of R. Rhabarbarum Extract On TNF-A, IL-6, And IL-10 Levels in Ethanol-Induced Gastric Ulceration.

Group	Pre-treatment	Dose	TNF-α (Pg/ml)	IL- 6 (Pg/ml)	IL-10 (Pg/ml)
G1	Negative Control	0.5% CMC	98	146	246
G2	Ulcer Control	0.5% CMC	530	345	107
G3	Omeprazole	20 mg/kg	128	137	238
G4	R. rhabarbarum	250 mg/kg	244	179	186
G5	R. rhabarbarum	500 mg/kg	131	149	234
Values represent serum concentrations of pro-inflammatory cytokines (TNF-α and IL-6) and the anti-inflammatory cytokine (IL-10). Ethanol-induced ulceration significantly increased TNF-α and IL-6 levels while reducing IL-10 compared to the negative control group ($p < 0.05$). Pre-treatment with R. rhabarbarum extract (250 and 500 mg/kg) significantly modulated these levels in a dose-dependent manner. The 500 mg/kg dose demonstrated an anti-inflammatory potency comparable to Omeprazole (20 mg/kg).					

4. Discussion

The present findings show that the methanolic extract of wild-grown Iraqi *Rheum rhabarbarum* exerts marked gastroprotective activity against ethanol-induced gastric injury. The high-dose extract reduced ulcer area by 82%, improved intragastric pH, with an overall effect close to that of omeprazole and preserved gastric mucus. This degree of protection is important because it suggests that the local Iraqi ecotype is not merely ethnobotanically relevant, but also pharmacologically active in a standard experimental ulcer model. For a regional journal readership, this is a noteworthy point: the study provides experimental support for a traditionally used Northern Iraqi plant and helps move its use from anecdotal practice toward evidence-based evaluation (Ahmed, 2016; Lanas and Chan, 2017).

A major strength of the present study is that the gastroprotective effect was supported by converging biochemical findings. Ethanol is known to cause rapid oxidative injury to the gastric mucosa and this was reflected in the ulcer control group by increased MDA and reduced CAT and SOD activities. Pretreatment with *R. rhabarbarum* significantly restored endogenous antioxidant defenses and attenuated lipid peroxidation indicating that protection was closely linked to redox stabilization. This interpretation is consistent with the literature showing that rhubarb species are rich in anthraquinone-derived constituents with recognized antioxidant, cytoprotective and phenolic properties (Cao *et al.*, 2017; Xiang *et al.*, 2020). Similarly, other ethanol-ulcer studies in which effective natural products have

been shown to reduce MDA while restoring antioxidant enzymes through pathways related to cellular stress resistance (Raish et al., 2021; Almasaudi et al., 2016; Arab et al., 2015).

The anti-inflammatory profile of the extract further strengthens its mechanistic relevance. In our model, ethanol markedly increased TNF-alpha and IL-6, while suppressing IL-10, whereas pretreatment with the extract reversed this pattern, especially at 500 mg/kg. Because TNF-alpha and IL-6 are central mediators of leukocyte recruitment, amplification of mucosal injury and endothelial dysfunction, their reduction suggests that the extract interferes with the inflammatory cascade rather than acting only as a physical mucosal coating. The rise in IL-10 is also significant because it points to restoration of anti-inflammatory balance within the injured gastric tissue. Similarly, cytokine modulation has been reported in other experimental gastroprotection studies and is considered a favorable indicator of tissue-level recovery (Almasaudi *et al.*, 2016; Ajeigbe *et al.*, 2017; Raish *et al.*, 2021).

The histopathological and immunohistochemical l results are consistent with these biochemical data and make the discussion stronger from a translational standpoint. Preservation of epithelial continuity and PAS-positive mucus indicates maintenance of the mucosal barrier, while reduced increased HSP70 and reduced Bax expression suggest that the extract promoted cytoprotection and limited apoptosis in stressed gastric cells. HSP70 is widely recognized as a stress-response protein that protects against protein misfolding and cell death, whereas Bax upregulation reflects activation of apoptotic injury pathways (Zorofchian *et al.*, 2014; Prithika and Balamurugan, 2019). The simultaneous improvement in macroscopic lesions, oxidative markers, cytokine balance and HSP70/Bax signaling, mucus preservation supports a multi-target mode of action rather than a single isolated mechanism. Particularly, this multi-layered protective pattern is valuable when positioning the manuscript for regional medical or pharmacology journals, because it shows that the reported activity is mechanistically coherent and not based on a single outcome measure.

From an Iraqi journal perspective, another important aspect is the local originality of the plant material. Geographic origin can influence the biological performance and phytochemical composition of medicinal plants and regional validation studies are therefore scientifically relevant rather than merely repetitive (Ahmed, 2016; Xiang *et al.*, 2020). By focusing on wild-grown *R. rhabarbarum* from Northern Iraq, the present study contributes locally grounded data that may be useful for future phytochemical standardization, fingerprinting, and comparison with Rheum materials from neighboring countries.

Limitations

At the same time, the findings should be interpreted in light of several limitations. First, this was a preliminary study performed in a single acute ethanol-induced ulcer model with a relatively small sample size. Second, the methanolic extract was not chemically standardized by chromatographic profiling, so the specific compounds responsible for the observed activity cannot yet be identified. Third, no molecular pathway assays such as Nrf2/HO-1, NF-kappaB, prostaglandin or mucus-related gene analyses were performed. Accordingly, future work should include detailed phytochemical characterization, pathway-oriented studies, dose-response refinement and additional ulcer models to strengthen translational confidence and support eventual development of an Iraqi plant-derived gastroprotective preparation (Xiang *et al.*, 2020; Raish *et al.*, 2021).

Conclusion

This study validates the traditional use of *Rheum rhabarbarum* in Northern Iraq. The local ecotype shows high efficacy in protecting gastric mucosa, comparable to standard pharmaceutical agents, likely through its anti-inflammatory and antioxidant pathways.

Conflict of Interest

The authors declare that they have no conflict of interest related to this work.

Contribution of Authors

Analysis and interpreted the experimental data: HH, EC. Manuscript Preperation: HH, EC, GM YCY, HC. Manuscript editing& review: YCY HC. Supervision of the project: EC

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Supplementary Tables

Supplement Table1: Effects of R. Rhabarbarumt Extract on Kidney Biochemical Parameters in Female Group of Rats.

Groups	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Urea (mmol/L)	Creatinine (mmol/L)
Normal range	136 - 145	3.5-5.0	96 - 106	6.4	35.3-70.7
Female Group Dosage					
Vehicle (1% Tween 20)	145.15 ± 0.52	4.36 ± 0.33	103 ± 0.45	5.25 ±1.15	34.18 ± 2.75
Plant extract 2.5 g/kg	144.05 ± 0.42	4.31 ± 0.04	104 ± 0.61	6.50 ± 0.74	33.63 ± 3.17
Plant extract 5 g/kg	143.11± 0.17	4.40 ± 0.23	105 ± 0.53	4.05 ± 0.33	35.52± 3.01
Values are expressed as mean ± standard error of the mean (SEM). There are no significant changes between treated and control groups ($p<0.05$). The results indicate that administration of *R. rhabarbarum* extract at doses of 2.5 and 5.0 g/kg did not cause any alterations in kidney function parameters, confirming the absence of nephrotoxicity in female rats.					

Supplementary Table2: Effects of R. Rhabarbarum Extract on Kidney Biochemical Parameters in Male Rats

Groups	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Urea (mmol/L)	Creatinine (mmol/L)
Normal range	136 - 145	3.5-5.0	96 - 106	6.4	35.3-70.7
Male Group Dosage					
Vehicle (1%Tween 20)	143.17 ± 0.43	4.33 ± 0.35	103.26 ± 0.45	5.85 ±1.14	35.13 ± 2.77
Plant extract 2.5 g/kg	142.13 ± 0.23	4.31 ± 0.08	104.10 ± 0.61	6.52 ± 0.72	34. 53 ± 3.16
Plant extract 5 g/kg	145.14 ± 0.19	4.42 ± 0.26	105.00 ± 0.53	6.09 ± 0.37	35.22 ± 3.04
Values are expressed as mean ± standard error of the mean (SEM). There are no significant changes between treated and control groups ($p<0.05$). The results indicate that administration of *R. rhabarbarum* extract at doses of 2.5 and 5.0 g/kg did not cause any alterations in kidney function parameters, confirming the absence of nephrotoxicity in female rats.					

Supplementary Table3: Effects of R. Rhabarbarum Extract on Liver Biochemical Parameters in Female Rats

Groups	Total protein (g/L)	Albumin (g/L)	ALP (IU/L)	ALT (IU/L)	AST (IU/L)
Normal range	60 - 83	34 - 54	30-130	10-40	50-150
Female Group					
Vehicle (1% Tween 20)	78.05 ± 2.50	12.31 ± 0.36	98.04 ± 1.46	35.22 ±1.16	197.12 ± 2.35
Plant extract 2.5 g/kg	77.35 ± 0.33	11.36 ± 0.24	95.24 ± 1.69	38.53 ± 1.72	192.68 ± 3.11
Plant extract 5 g/kg	76.16 ± 0.18	13.42 ± 0.25	92.75 ± 1.58	33.07 ± 1.36	194.50 ± 3.31
Values are expressed as mean ± standard error of the mean. There are no significant changes between groups. Significant value at $p<0.05$. The results show that administration of plant root extract at doses of 2.5 and 5.0 g/kg did not produce any significant changes in total protein, albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), or aspartate aminotransferase (AST) levels in serum when compared with the vehicle control group ($p>0.05$). All biochemical values remained within the normal physiological range, implying that the plant extract has no effects on the hepatoxicity at the tested doses.					

Supplementary Table4: Effects of Plant Root Extract on Liver Biochemical Parameters in Male Rats

Groups	Total protein (g/L)	Albumin (g/L)	ALP (IU/L)	ALT (IU/L)	AST (IU/L)
Normal range	60 - 83	34 - 54	30-130	10-40	50-150
Dose in male					
Vehicle (1% Tween 20)	68.25 ± 2.53	13.32 ± 0.38	105.06 ± 1.49	48.28 ±1.12	203.32 ± 2.37
Plant extract 2.5 g/kg	67.15 ± 0.37	12.38 ± 0.22	110.23 ± 1.42	52.51± 1.76	207.62 ± 3.15
Plant extract 5 g/kg	66.36 ± 0.17	12.44 ± 0.28	108.15 ± 1.27	45.23 ± 1.33	211.52 ± 3.35
Values are expressed as mean ± standard error of the mean. There are no significant changes between groups. Significant value at $p<0.05$. The results show that administration of plant root extract at doses of 2.5 and 5.0 g/kg did not produce any significant changes in total protein, albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), or aspartate aminotransferase (AST) levels in serum when compared with the vehicle control group ($p>0.05$). All biochemical values remained within the normal physiological range, implying that the plant extract has no effects on the hepatoxicity at the tested doses.					