

Chemical constituents and anti-gastric ulcer activity of *quercus infectoria* galls in an ethanol-induced gastric ulcer model

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Abstract

Background and objective: *Quercus infectoria* galls (*Q.infectoria*), rich in polyphenols, have been traditionally used to treat gastric disorders. This study aimed to evaluate their phytochemical composition, antioxidant capacity, anti-inflammatory, anti-gastric ulcer efficacy, and safety in an ethanol-induced gastric ulcer model.

Methods: Ethanol extracts were obtained using ultrasonic and reflux methods. Quantitative estimation of phenols, flavonoids, and tannins, and antioxidant activity (DPPH assay) were performed. HPLC analysis was conducted to identify major phenolic constituents. Anti-ulcer efficacy was assessed in rats with ethanol-induced gastric ulcers, followed by measurement of cytokines (TNF- α , IL-1 β) and biochemical safety parameters.

Results: *Q.infectoria* galls showed high levels of phenolics, flavonoids, and tannins, and exhibited vigorous DPPH scavenging activity (IC₅₀: 43.88 μ g/mL). HPLC confirmed gallic acid, chlorogenic acid, and kaempferol as major constituents. The extract significantly reduced ulcer index, gastric acidity, and inflammatory markers, with effects comparable to esomeprazole and no observed toxicity.

Conclusion: These findings indicate that galls of *Q.infectoria* possess anti-ulcer potential, mediated by their antioxidant and anti-inflammatory properties. It may serve as a safe, natural alternative for managing gastric ulcers.

Keywords: Anti-gastric ulcer, DPPH assay, HPLC, *Quercus infectoria*.

Introduction

Plants have long been an integral part of healthcare in various cultures. This is primarily due to the presence of active phytochemicals (1). *Q.infectoria* is one of the plants in the Fagaceae family with medicinal value. Gall oak is a small tree

or shrub, with a height of approximately 2.5 meters. It is available in Cyprus, Greece, Turkey, Egypt, Iraq, and Iran (2). Galls available on this species' branches are caused by the egg-laying activity of the gall wasp *Cynips gallae tinctoriae* (3). The galls have different

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colours, such as olive green, white, brown, olive green-grey, or dark blue-green (4). Gallo tannin is the principal constituent of these galls, accounting for almost 50–70% of the amount. It also contains many other components like trace amounts of unbound ellagic acid, gallic acid (2-4%), starch, and sugars (5). Pharmacologically, *Q.infectoria* galls have been known for a broad spectrum of effects, including antioxidant, antimicrobial (including antibacterial, antifungal, and antiviral), larvicidal, natural astringent, antidiabetic, anti-inflammatory, anti-diarrheal, and wound-healing properties (6). Gastric ulcer issues are a worldwide problem, with an estimated 10% of the population affected (7). Gastric ulcers arise from an imbalance of the stomach's aggressive and protective factors (8). Critical stressors comprise alcohol, non-steroidal anti-inflammatory drugs (NSAIDs), smoking, and *H. pylori* infection (9). Ethanol can cause both dehydration and damage to the gastric tissues, resulting in mucosal oedema and hemorrhage (10). Currently, proton pump inhibitors, H₂-receptor antagonists, and antibiotics aimed at eliminating *H. pylori* infection are the standard treatment for gastric ulcers (11). Nonetheless, there are many complications with the continued use of these acid-suppressing medications. Due to concerns like these, interest is shifting toward safer options. Research suggests that some medicinal plants may be effective, natural

treatments for ulcer healing with fewer side effects. Medicinal plants are considered potential candidates due to their low adverse effects (12). In the Kurdistan region, galls of *Q.infectoria* are noted for their anti-gastric ulcer properties. Despite the wide use of the plant, scientific reports that support its traditional uses remain limited. Earlier research on *Q.infectoria* has primarily focused on its antibacterial and antioxidant properties. For this purpose, the current project focuses on the potential curative effects of *Q.infectoria* galls on gastric ulcers induced by ethanol exposure in a rodent model. A relevant phytochemical study also assesses the extract's safety, antioxidant, anti-inflammatory properties, and anti-ulcer activity.

Collection, Extraction, and Quantitative Study of *Q. infectoria*

Galls of *Q.infectoria* have been collected from the mountainous regions of the Kurdistan Region in September 2024. Botanists from Salahaddin University identified the species, and a voucher specimen was deposited (H-11). Galls were air-dried in the shade, cleaned, and stored in sealed containers. Powdered galls of *Q.infectoria* were first defatted with petroleum ether (Chemolab, Belgium). The subsequent steps involved extracting the defatted material with 80% ethanol (Chemolab, Belgium) utilizing two methods: Ultrasonic-Assisted Extraction (Labtech PowerSonic405, South Korea) at 40°C

for 60 minutes and conventional reflux extraction for 3 hours. After concentrating under reduced pressure with a rotary evaporator (BÜCHI, Switzerland), the extract was kept at 4°C until further examination. Quantitative determinations were carried out to quantify the total flavonoid content (TFC), total phenolic content (TPC), and total tannin content (TTC). The aluminum chloride colorimetric method with quercetin as a standard was used to determine TFC. The TPC procedure involves the reaction of the Folin-Ciocalteu mixture with the extract, which uses gallic acid as a reference compound. TTC was determined by the gallic acid equivalent of a modified Folin–Ciocalteu assay. Spectrophotometric (Shimadzu UV-1900i) measurements of all absorbances were done at 415, 760, and 725 nm, respectively (13, 14).

HPLC Analysis

Phenolic compounds from *Q.infectoria* extract were separated with High Performance Liquid Chromatography (HPLC) (SYKAM, Germany) using a C18-ODS analytical column with dimensions of 250 mm × 4.6 mm and 5 µm particle size. The sample was centrifuged for filtration, and a 100µL aliquot was injected into the system. The mobile phase consisted of two solvents in distilled water: A, 95% acetonitrile (Scharlau, Spain), and B, 0.01% trifluoroacetic acid (Scharlau, Spain). The analysis was done at a constant

flow rate of 1.0 mL/min with a gradient elution profile starting at 10% A for 5 minutes, then increasing to 25% A for 5-7 minutes, then 40% A from 7–13 minutes. The column was then returned to starting conditions. Eluted phenolic compounds were detected with a UV-Vis detector set at 278 nm (15).

In vitro antioxidant

The free radical neutralizing effect of the extract was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. Several concentrations of the extract were individually reacted with an ethanolic DPPH solution (Merck, Germany) and kept in the absence of light to prevent photodegradation. Following the incubation period, the decline in absorbance at 517 nm was recorded spectrophotometrically. As a standard comparator, alpha-tocopherol (Sigma-Aldrich, USA) was employed to evaluate the extract's efficacy. The percentage inhibition of the DPPH radicals was determined, and the concentration that achieved 50% scavenging activity (IC₅₀) was used to express the antioxidant strength of the sample, using the formula:

$$\% \text{ inhibition} = [(A_0 - A_1) / A_0] \times 100.$$

A₀ denotes the absorbance of the control, and A₁ is the absorbance of the test sample (13).

Biological activity

Test Animals

Wistar rats were sourced from the Hawler Medical University animal house. All animals were maintained under standardized laboratory settings, including a room temperature of $23 \pm 1^\circ\text{C}$, humidity levels ranging from 50% to 70%, and a 12-hour alternating light and dark cycle. The rats had unrestricted access to standard rodent feed and clean drinking water throughout the experiment. The study was ethically approved by the Ethical Committee of the College of Pharmacy/ Hawler Medical University under the reference number: HMU/CPH/29162024-46, dated 29 June 2024.

Acute Oral Toxicity Study

The acute toxicity study was performed according to OECD Guideline 423 (16). Based on OECD, adult female Wistar rats weighing between 200-250 g ($n=24$) were randomly assigned to four groups of six animals. The control group received a vehicle only; the remaining were given a single oral dose of the plant extract at 1000, 1500, and 2000 mg/kg body weight via oral gavage. Animals were fasted overnight with water allowed ad libitum before administration. Immediately after treatment and four hours post-treatment, the rats were actively monitored for signs of toxicity, behavioral changes, or survival.

Subsequently, they were monitored over 14 days. Parameters such as nutritional intake, hydration, body mass, and general actions were recorded daily.

Study Design

This study was designed to assess the curative effects of *Q.infectoria* extract on ethanol-induced gastric ulcers in rats over a one-month period. Gastric ulcers were induced by administering absolute ethanol (99.9%) at a dose of 5 mL/kg via oral gavage (17). This model simulates pre-existing gastric damage, with treatment initiated one hour after ulcer induction and continued once daily for 28 successive days. Adult male Wistar rats (180–220 g) were randomly divided into five groups ($n = 6$): normal control (no ethanol; received distilled water only), ulcer control (received ethanol only), standard drug (received ethanol + esomeprazole, 20 mg/kg/day), low-dose extract (received ethanol + *Q.infectoria* extract, 250 mg/kg/day), and high-dose extract (received ethanol + *Q.infectoria* extract, 500 mg/kg/day) (17).

On day 29, all animals were sedated with an intraperitoneal injection of ketamine 10% and xylazine 2% (Alfasan, Holland). Blood samples were then collected by cardiac puncture for biochemical study, and stomachs were collected for macroscopic examination. For long-term safety evaluations, lipid profile, renal, and hepatic functions were evaluated after the treatment period.

Anti-Gastric Ulcer Activity

The ethanol-treated animals were evaluated for the anti-ulcer and anti-inflammatory effects of *Q.infectoria*. During the post-mortem, the stomach was cut open along the greater curvature, and distilled water was added to flush the gastric contents. Their stomachs were pinned flat on a corkboard with the glandular mucosa side upwards. The mucosa was looked at with the naked eye and a 10x magnifying lens. The grading scale for ulcers was from 0 to 5 based on how severe it was, their average score of ulcers per animal was termed the ulcer index (UI), where $UI = UN + US + UP/10$; with UN representing the mean number of ulcers per animal, US the mean severity score, and UP the percentage of animals that had ulcers. The equation calculated the inhibition of ulcers (18):

$$\% \text{ Inhibition} = (\text{Ulcer index control} - \text{ulcer index test}) / \text{ulcer index control} \times 100$$

The PH of gastric juice was measured directly with a digital pH meter (Sartorius, Germany), and its value was recorded. The clear gastric juice sample (1 mL) was divided to assess total acidity, and one part was set aside (1 mL) containing distilled water. This solution was titrated with 0.01 N NaOH (Biochem, France) using two drops of phenolphthalein (Scharlau, Spain) as an indicating marker. The endpoint was identified as faint pink (17). Total acidity

was computed through the equation (19):

$$\text{Total Acidity} = \text{volume of NaOH} \times \text{Normality of NaOH} \times 100 / 0.1$$

Serum levels of inflammatory cytokines, including tumor necrosis factor-alpha (TNF- α) and interleukin-1 beta (IL-1 β), were computed using ELISA kits from Sunlong Biotech Co., Ltd, China, to reveal *Q.infectoria* galls' immunomodulatory action. The application steps were carried out according to the manufacturer's instructions.

Statistical analysis

Statistical analyses were done using Microsoft Excel 2019 and SPSS version 26. Figures and tables were presented using mean values followed by the Standard Deviation (SD). A one-way analysis of variance (ANOVA) was performed for experimental group distinctions between the defined levels. The critical level of statistical significance was $P < 0.05$.

Results

Extraction and Phytochemical Composition

The yield from ultrasonic-assisted extraction (UAE) was 35.32%, while that from the conventional reflux method (RE) was higher at 42.5% (Table 1). Quantitative screening of *Q.infectoria* galls extracts. The highest total phenolic content was noted, followed by tannins and a lower level of flavonoids (Table 2).

HPLC Analysis

The HPLC chromatogram of *Q.infectoria* extract shows 10 well-resolved peaks representing distinct phytochemical compounds (Figure 1). These compounds were identified based on their retention times with reference

samples, the standard compounds and their retention times are: apigenin (2.25 min), caffeic acid (3.85 min), catechin (4.08 min), chlorogenic acid (5.79 min), kaempferol (6.00 min), ellagic acid (7.95 min), quercetin (9.65 min), rutin (9.91 min), ferulic acid (10.25 min) and gallic acid (12.33 min). The regions of the peaks also differ, indicating a diverse composition of the extract. From the quantitative analysis, the concentrations of the above compounds were between 51.6 and 105.9 µg/mL, as detailed in Table 3. It is also important to note that the measured peak areas were considerably different, which indicates differences in the concentration of the individual components.

Table 1. Extraction Yield and Physical Characteristics

Extraction methods	Ultrasonication	Ordinary reflex
Percentage of yield	35.32%	42.5%
Color		Olive green
Consistency		Solid powder

Table 2. Phytochemical Content and Antioxidant Activity (IC₅₀) of *Q.infectoria*

Samples	TPC ^a (GAE µg/mg)	TFC ^a (QE µg/mg)	TTC ^a (GAE µg/mg)	IC ₅₀ ^a (µg/mL)
<i>Q. infectoria</i>	386.44±2.17	1.023±0.015	178.69±4.82	43.88± 0.04
α- tocopherol	40.87±0.35

Values are shown as mean ± SD by using the average of three (n=3)

a: TPC refers to total phenol content; TFC refers to total flavonoid content; TTC refers to total tannin content; IC₅₀ refers to the minimal inhibitory concentration to counteract 50% of free radicals

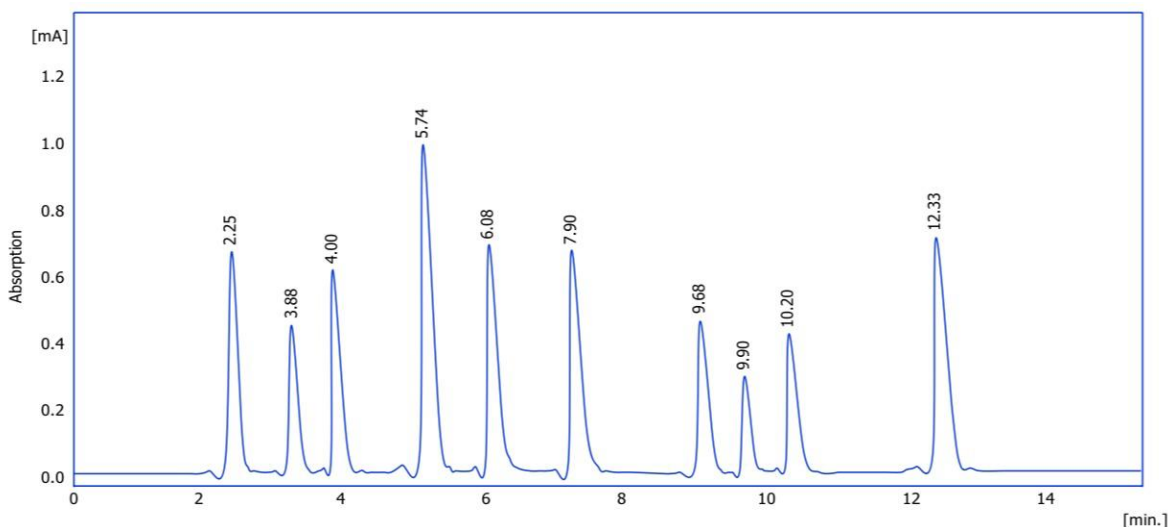
Table 3. Quantitative analysis for 80% ethanolic extract of *Q. infectoria* galls by HPLC

No	Compound name	RT ^a standard [min]	RT sample [min]	Area [mAU.s] ^b	Height [mAU]	Concentration (ppm) ^c
1	Apigenin	2.25	2.25	5985.08	631.45	74.6
2	Caffeic acid	3.85	3.88	3254.99	411.21	64.1
3	Catechin	4.08	4.00	5014.98	574.14	66.5
4	Chlorogenic acid	5.79	5.74	9851.14	974.41	98.8
5	Kaempferol	6.00	6.08	6022.54	701.25	85.9
6	Ellagic acid	7.95	7.90	6231.49	700.11	81.4
7	Quercetin	9.65	9.68	4158.79	496.32	60.8
8	Rutin	9.91	9.90	2155.64	386.74	77.4
9	Ferulic acid	10.25	10.20	3698.08	374.12	51.6
10	Gallic acid	12.33	12.33	7458.92	634.09	105.9

a. Retention time in minutes.

b. Milli absorbance units multiply by seconds to measure the area under the curve.

c. Results are presented as parts per million ($\mu\text{g/mL}$).

**Figure 1.** Chromatogram of 80% ethanolic extract of *Q. infectoria* galls.

In Vitro Antioxidant Activity

As sample concentration increases, extract percentage inhibition also rises, validating *Q.infectoria's* considerable antioxidant activity. The DPPH radical scavenging assay further confirmed this. The extract scavenging effect at 100 µg/mL achieved a value over 93%, higher than the inhibition presented by α-tocopherol, 85 % (Figure 2). For *Q.infectoria* extract, the IC₅₀ value (the concentration required to inhibit 50% of DPPH radicals) was 43.88 ± 0.04 µg/mL, while for α-tocopherol it was 40.87 ± 0.35 µg/mL, indicating that both possess similar oxidative capacities (Table 2).

Acute Toxicity Test and Long-term Safety of *Q. infectoria* galls Extract

There were no notable toxic effects at doses of 1000 mg/kg and 1500 mg/kg. Some mild lethargy, reduced activity, and slight tremors at high doses. These symptoms were temporary and lasted

only for a few hours. No fatalities occurred at varying doses. After oral treatment with *Q.infectoria* extract, the results showed no adverse changes in liver function, as indicated by consistent ALT, AST, and ALP levels in both groups compared to the control. The total and direct levels of bilirubin also remained within physiological limits, which, along with the ALT and AST, suggests the integrity of the liver. Serum markers of renal function, such as urea, creatinine, and uric acid, also did not change significantly, suggesting no nephrotoxicity. No significant change in the lipid profile with treatment was observed in triglycerides, total cholesterol, LDL, and HDL, indicating no alteration. Findings suggest both low and high doses of *Q. Infectoria* extract were tolerated without signs of systemic or metabolic toxicity, as shown in Table 4, thus verifying its safety for prolonged usage.

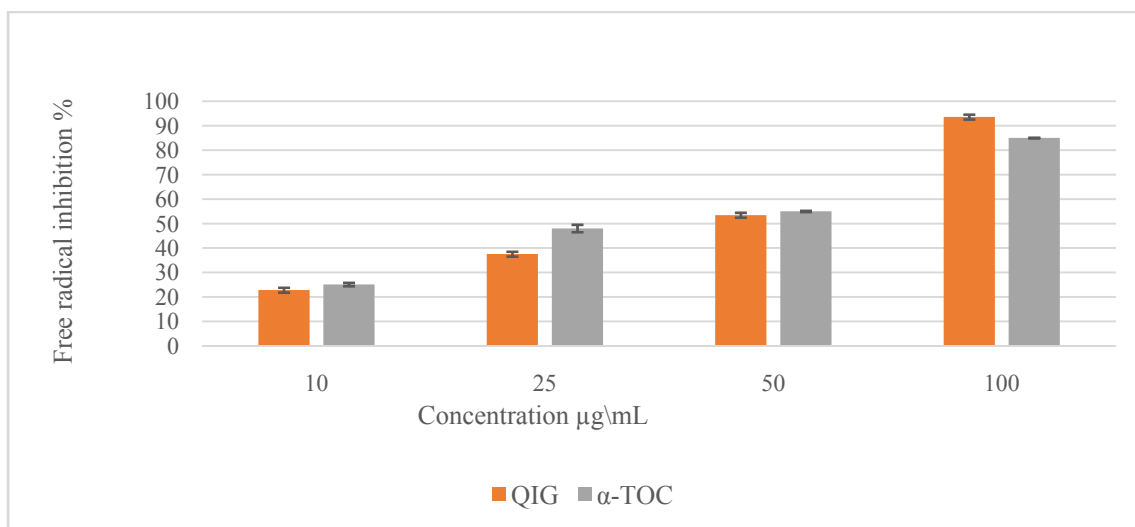


Figure 2. Dose-response DPPH assay of *Q.infectoria* gall extract antioxidant activity. QIG stands for *Q.infectoria* gall, α-TOC stands for alpha-tocopherol. All tests were done in triplicate (n=3). Bar graphs show averages of three replicates

Table 4. Biochemical and Metabolic Safety Parameters of *Q. infectoria* Galls Extract in Rats (n=6)

Parameters	Healthy control	<i>Q. infectoria</i> galls (250 mg/kg)	<i>Q. infectoria</i> galls (500 mg/kg)	P-value
Alanine aminotransferase (U/L)	40.36±1.25	39.25±0.35	44.23±3.70	0.079
Aspartate aminotransferase(U\L)	71.56±6.41	72.86±2.12	78.20±3.75	0.234
alkaline phosphatase (U/L)	136.47±6.98	139.02±6.00	142.31±4.52	0.519
Total Bilirubin (mg\dl)	0.35±0.25	0.50±0.05	0.6±0.1	0.193
Direct Bilirubin (mg/dL)	0.1±0	0.15±0.05	0.2±0.1	0.244
Urea(mg\dl)	30.15±3.25	31.11±0.87	35.10±4.9	0.252
Creatinine(mg\dl)	0.445±0.017	0.41±0	0.57±0.07	0.159
Uric acid(mg\dl)	2.9±0.8	3.3±0.2	4.36±0.12	0.059
Triglycerides (mg\dl)	49.3±5.9	48.65±6.1	45.95±2.05	0.702
Cholesterol (mg/dL)	70.9±1.4	72.5±4.2	66.1±5.23	0.210
Low Density Lipoprotein (mg/dL)	63±7	62.33±9.5	55.33± 1.95	0.382
High Density Lipoprotein (mg/dL)	40.64±3.11	41.03±1.40	47.3±3.45	0.081

The results were introduced as mean ± SD; the Difference between healthy animals and treated groups was insignificant at a P-value >0.05.

Anti-Gastric Ulcer Activity

The ulcer control group had an ulcer index of 9.03 ± 0.21 , showing severe damage to the gastric mucosa with ulceration following administration of absolute ethanol (5 mL/kg) as described in Figure 3 and Table 5. *Q.infectoria* extract at doses of 250 mg/kg and 500 mg/kg improved this significantly to 5.23 ± 0.26 and 2.22 ± 0.54 , respectively. *Q.infectoria* high dose is similar to the effect of esomeprazole (P-value = 0.401). The curative rates were 42.08% (250mg/kg) in the lower dose of *Q.infectoria* and 75.45% (500mg/kg), and esomeprazole 80.62% (P = 0.153).

Regarding gastric pH and total acidity, all parameters significantly improved relative to the ulcer control group (No meaningful differences were seen in gastric PH between *Q.infectoria* 500 mg/kg and esomeprazole; P-value = 0.678). Stomach acidity was pointedly elevated in the ulcer-positive group and linked to the treatment groups, where it was markedly reduced. No statistically significant difference was observed in the esomeprazole and high-dose treatment groups (P = 0.315). Following treatment with the extract, gastric pH increased while total acidity decreased in a dose-response fashion (Table 5).

Table 5. Effects of *Q.infectoria* Gall Extract on Gastric Ulcer Parameters in Ethanol-Induced Ulcerated Rats

Groups	Ulcer Index	Curative Rate	Gastric PH	Total Acidity (mEq\L)
Ulcer control group	9.03 ± 0.21	1.96 ± 0.035	70.4 ± 1.13
Esomeprazole group (20mg\ kg)	1.75 ± 0.20	80.62	5 ± 1	31.50 ± 1.05
<i>Q.infectoriagall</i> (250mg\kg)	5.23 ± 0.26	42.08	3.26 ± 0.17	45 ± 2.94
<i>Q.infectoria gall</i> (500mg\kg)	2.22 ± 0.54	75.45	$4.60^{**} \pm 0.12$	36.67 ± 4.50
P-value	P < 0.001	P < 0.001	P < 0.001	P < 0.001

Values were reported as mean \pm SD (n = 6); * Different from ulcer control statistically, P = 0.048. ** Different from ulcer control statistically, P = 0.002 (ANOVA followed by Bonferroni post hoc test).

Q.infectoria galls extract treatment markedly decreased serum levels of TNF- α and IL-1 β . The high-dose group (500 mg/kg) still suppressed TNF- α the most, where the difference from esomeprazole was not statistically

significant ($P = 0.089$). After both doses of *Q.infectoria*, there was no statistical difference in the level of IL-1 β when compared with standard treatment ($P = 0.904$). The variation in results is presented in Table 6.

Table 6. Anti-inflammatory effect of *Q.infectoria* galls.

Groups	TNF alpha* (pg/mL)	P value	IL-1 beta* (pg/mL)	P value
Ulcer control group	67.36 \pm 2.75	23.59 \pm 2.09
Esomeprazole group	51.37 \pm 4.71	0.022	12.27 \pm 2.02	0.001
<i>Q.infectoria</i> gall (250mg\kg)	50.35 \pm 2.99	0.018	12.70 \pm 1.21	0.001
<i>Q.infectoria</i> gall (500mg\kg)	43.03 \pm 3.80	0.003	10.37 \pm 1.19	< 0.001

Values are expressed as mean \pm SD (n = 6). Differences between ulcer control and treatment groups were statistically significant at $P < 0.05$ (ANOVA followed by Bonferroni post hoc test). *TNF- α : Tumor necrosis factor alpha; IL-1 β : Interleukin-1 beta.

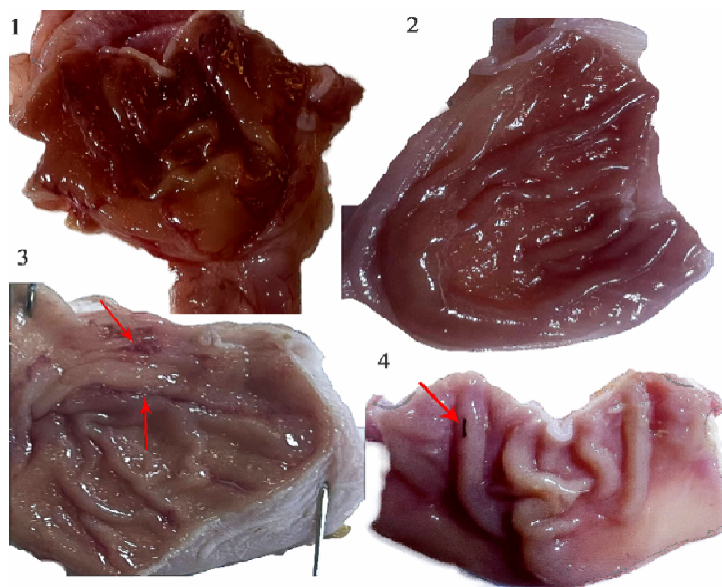


Figure 3. Macroscopic appearance of the gastric mucosa; 1. Ulcer positive group; 2. Esomeprazole group; 3. Low dose *Q. infectoria*; 4. High dose *Q. infectoria*

Discussion

This study is the first to investigate the accepted traditional use of *Q.infectoria* in managing pre-existing gastric ulcers, along with its mechanisms of action through multiple biochemical parameters, including ulcer index, gastric pH, total acidity, and pro-inflammatory cytokines (TNF- α and IL-1 β). It is worth noting that differences in the chemical composition, antioxidant activity, and healing effects of *Q.infectoria* may be influenced by geographical growing conditions, as the plant material used in this study was sourced from the Kurdistan region. In the present study, *Q.infectoria* galls were extracted using both reflux and ultrasound-assisted techniques to increase the recovery of bioactive compounds and compare the efficacy of conventional and modern extraction methods. The results were consistent with Lana's previous findings, which showed that RE has a higher extract yield than UAE (20). This is explained by the fact that longer extraction time at maintained elevated temperatures improves solvent penetration into the plant matrix and extraction of bound high-molecular-weight compounds like tannins and flavonoids (21). Using a spectrophotometric approach, it was noted that the extract contains a significant amount of tannins and phenols, pertaining to the polyphenolic nature of *Q.infectoria* galls, as proved by earlier research groups. HPLC Analysis

also provided evidence for identifying several key bioactive constituents, gallic acid, chlorogenic acid, ellagic acid, kaempferol, and others, as the dominating constituents. The antioxidant potential was validated with the DPPH assay, where the *Q.infectoria* extract increased free radical inhibition dose-dependently. A low IC₅₀ value of *Q.infectoria* extract indicates strong antioxidant potential, as little extract is needed to scavenge half of the DPPH radicals. Our results agree with earlier findings by another research group, who reported that *Q.infectoria* extract contains high concentrations of phenols and flavonoids, identified and quantified by HPLC. They found that the extract exhibits strong antioxidant activity, as indicated by its low IC₅₀ value (47 μ g/mL) (22). The gastric ethanol-induced mucosal injury model in rats is common for testing the gastroprotective effect because it is similar to human gastric ulcer (23). The neutrophil response to the stomach's exposure to ethanol is a rapid influx into the area of injury. They damage the stomach lining through the oxidative stress caused by free radicals and the release of inflammatory enzymes (24). Ethanol is known to increase the ulcer index and decrease stomach PH and acidity, which we found to correspond with previous works (25). After treatment with *Q.infectoria*, the ulcer index decreased while pH increased, supporting that the extract aided in protecting and healing the stomach lining. The higher cure rate

in treatment groups indicates that the extract has the potential to assist in the healing of gastric ulcers. Increased TNF- α and IL-1 β levels were documented after ethanol exposure, which suggests that the compound activates a strong inflammatory response in the gastric mucosa (26). Our research demonstrates that *Q.infectoria* diminished gastric inflammation by dampening the action of pro-inflammatory cytokines. We also found that *Q.infectoria* had increased TNF- α and IL-1 β levels reduction compared to esomeprazole treatment, but the difference was not statistically meaningful ($P > 0.05$). The ulcer-healing activity of *Q.infectoria* is due to the presence of polyphenolic compounds such as tannins, which aid in forming a protective barrier to the damaged tissues and promote healing of the ulcer. Besides that, tannins help reduce inflammation in the ulcerated area partly through mild vasoconstriction, which decreases capillary leakage, limits the release of inflammatory mediators, and helps reduce tissue swelling (27). It is well documented that phenolic acids can interact with and neutralize free radicals via hydrogen donation and redox reactions. For instance, Gallic acid has been noted in the literature to possess antihistaminic properties, which decrease the gastric acid secreted and help prevent ulcer formation. It also acts on pro-inflammatory cytokines such as TNF- α and IL-1 β , thus moderating the inflammation in the

gastric mucosa. Chelating iron protects tissues from oxidative damage and prevents the formation of reactive oxygen species (28). Contemporary studies done with animals have revealed that chlorogenic and ellagic acids have pronounced gastroprotective potential against ethanol-induced gastric ulcers. These compounds have shared mechanisms that include antioxidant, anti-inflammatory, and mucosal protective effects (29, 30). Prior animal studies have demonstrated that kaempferol, a flavonoid, causes protective changes in the stomach in ethanol ulcer-induced models. Such protection is assumed to stem from its actions of preserving gastric mucous glycoproteins, inhibiting the neutrophil myeloperoxidase (MPO) activity, reducing pro-inflammatory cytokines, and increasing nitric oxide (NO) (31). Proposed acute toxicity study implies that *Q.infectoria* is relatively safe in low and moderate doses but causes minor and reversible symptoms in high doses. These results correlate with previous findings on the toxicity of *Q.infectoria* (32). The safety measures taken on the plant involved assessing the subject's kidney, liver, and lipid profile. The absence of changes in these parameters means that the substance did not disturb lipid metabolism or cause disorders associated with dyslipidemia. Stable levels of lipids indicate that there were no negative effects on cardiovascular health and metabolism (33). Regarding the liver, results did not

show any significant changes, which does not support the assertion that the extract promotes hepatic toxicity. Moreover, total and direct bilirubin levels were within normal limits, suggesting that the liver's capability to manage and metabolize waste was intact (34). The tests conducted on kidney functions did not reveal any injuries or reduced filtration capacity, thereby establishing the substance's safety concerning renal health (35).

Conclusion

This research demonstrates that *Q.infectoria* galls can effectively alleviate ethanol-induced gastric ulcers. HPLC analysis showed that the extract bears critical bioactive constituents, including phenolic acids and flavonoids. These compounds counteract oxidative free radicals and reduce inflammatory cytokines, which assist in healing the stomach. At higher dosages, *Q.infectoria* was as effective as esomeprazole in treating gastric ulcers but did not damage the liver or kidneys. However, additional inspection is needed to understand *Q.infectoria* galls' mechanisms of action. Further studies should target how the compound modulates oxidative stress and inflammation at the cellular level to validate its use as a natural remedy for gastric ulcers.

Competing interest

The authors declare that they have no competing interests.

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