Phyto-Pharmacological Evaluation of *Acacia seyal* Leaf Extract for the Treatment of Peptic Ulcer

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Abstract

The present study explores the anti-ulcer efficacy of *Acacia seyal* leaf extract (ASLE) in Wistar rats using the pylorus ligation-induced ulcer model. Five groups of rats were utilized: normal control, positive control, standard (Ranitidine, 20 mg/kg p.o.), and two test groups receiving ASLE at low (250 mg/kg p.o.) and high (500 mg/kg p.o.) doses. Histopathological analysis of stomach tissues was performed to evaluate ulceration and cellular changes. Results showed a significant reduction in ulcer index in the ASLE-treated groups, particularly at the higher dose of 500 mg/kg, indicating a dose-dependent gastroprotective effect. The standard drug group (Ranitidine) exhibited the most pronounced reduction in ulceration, along with well-organized gastric cell structures. Positive control animals showed severe ulceration and cellular damage. Histopathological findings further demonstrated that ASLE treatment preserved the gastric mucosa with fewer lesions and more intact cell architecture. The anti-ulcer activity of ASLE may be linked to its rich phytochemical content, including alkaloids, flavonoids, saponins, and tannins, which are known for their antioxidant and anti-inflammatory properties, potentially reducing gastric acid secretion and enhancing mucosal defenses.

Keywords

Acacia seyal, pylorus ligation, anti-ulcer activity, peptic ulcer, gastric mucosa, phytochemicals, histopathology, Ranitidine, gastroprotective.

1. Introduction

An ulcer is a rupture in the skin or mucous membrane that leads to pus formation, tissue disintegration, and necrosis of epithelial cells (1). Peptic ulcers, in particular, involve the breakdown of the mucosal lining of the stomach and/or duodenum due to an imbalance between aggressive factors, such as gastric acid and pepsin, and the protective mucosal defenses. Several factors, including infection by *Helicobacter pylori*, prolonged use of nonsteroidal anti-inflammatory drugs (NSAIDs), and excessive alcohol consumption, contribute to the development of peptic ulcers (2). Although synthetic drugs are available to treat ulcers, they often come with side effects, prompting the exploration of safer, plant-based alternatives.

Acacia seyal possesses medicinal properties similar to those of *Acacia nilotica*, as both species have been traditionally used for their anti-inflammatory, antimicrobial, and antioxidant effects. *Acacia seyal*, commonly known as the gum arabic tree (3), is a medicinal plant traditionally used to treat various ailments, including gastrointestinal disorders. It contains bioactive compounds like tannins, flavonoids, alkaloids, and phenolic compounds, which have been shown to exhibit anti-ulcer, antimicrobial, antioxidant, and anti-inflammatory properties (3). These compounds are particularly effective in enhancing mucosal defense, neutralizing free radicals, and inhibiting the growth of *H. pylori*, making *Acacia nilotica* a promising candidate for the treatment of peptic ulcers (4).

This research aims to investigate the therapeutic potential of *Acacia seyal* in ulcer management, focusing on its pharmacological properties, mechanisms of action, and bioactive constituents. By exploring this natural alternative, the study hopes to contribute to the development of safer, cost-effective treatments for peptic ulcer disease.

2. Materials and Methods

2.1 Collection of Plant Material

Acacia seyal leaves were collected from Prayagraj, Uttar Pradesh, India, during the months of December and January. The plant was identified and authenticated by a taxonomist at the Botanical Survey of India, Prayagraj, with a herbarium deposition (vide number: B.S.I/C.R.C./2023-24/774). The fresh foliage was cleaned by thorough washing to remove dust, then spread in thin layers on drying trays and left in the shade for two weeks. After complete drying, the leaves weighed 1200 g and were ground into a fine powder using a mechanical grinder. The powder was sieved through mesh size #40 for consistency .

2.2 Extraction Method

The powdered leaves (50 g) were first defatted using petroleum ether for 48 hours, followed by continuous Soxhlet extraction at 50°C using a hydroalcoholic solvent (ethanol, 70:30). The extract was concentrated under reduced pressure using a rotary evaporator and stored in airtight containers at 4°C for further analysis (5) .

2.3 Phytochemical Screening of Extracts

The ethanolic extract was subjected to qualitative phytochemical screening to determine the presence of various bioactive compounds. The following tests were conducted to identify key classes of phytochemicals (6-9):

(a) Wagner's Test for Alkaloids

In this test, 4 ml of the extract was combined with 3 drops of Wagner's reagent, and the mixture was allowed to stand for 5 minutes. The formation of a reddish-brown precipitate indicated the presence of alkaloids.

(b) Sodium Hydroxide Test for Flavonoids

Approximately 0.2 g of the extract was dissolved in a mixture of cold dilute sodium hydroxide and dilute hydrochloric acid. The absence of yellow coloration indicated the presence of flavonoids.

(c) Copper Acetate Test for Terpenes

Five milliliters of the extract were mixed with 12 drops of copper acetate solution. The appearance of a beryl green color signified the presence of terpenes.

(d) Salkowski Test for Steroids

To 5 ml of the extract, 2.5 ml of chloroform and 2.5 ml of concentrated sulfuric acid were added. Red fluorescence in the chloroform layer and greenish-yellow fluorescence in the acid layer indicated the presence of steroids.

(e) Foam Test for Saponins

Three milliliters of the extract were shaken vigorously with 2 ml of water for 10 minutes. The formation of a stable foam indicated the presence of saponins.

(f) Ferric Chloride Test for Phenolics

Half a milliliter of the plant extract was heated with 5 ml of distilled water for 10 minutes. Three drops of 10% ferric chloride were added to 2 ml of the filtrate. A greenish-blue or violet color indicated the presence of phenolics.

(g) Lead Acetate Test for Tannins and Phenols

Four milliliters of the extract were mixed with 4 ml of lead acetate solution. The formation of a white precipitate confirmed the presence of tannins and phenols.

(h) Borntrager's Test for Anthraquinone Glycosides

Two milliliters of the extract were boiled and filtered with diluted sulfuric acid. The filtrate was shaken with chloroform, and after separation, ammonia was added. A pink to red color change in the ammoniacal layer indicated the presence of anthraquinone glycosides.

(i) Fluorescence Test for Coumarin Glycosides

Two milliliters of the extract were mixed with 1N sodium hydroxide solution. The presence of coumarin glycosides was confirmed by observing a bluish-green fluorescence.

(j)Keller-Killiani Test for Cardiac Glycosides

A mixture of glacial acetic acid, ferric chloride, and concentrated sulfuric acid was added to the extract dissolved in water. The formation of a brown ring at the interface confirmed the presence of cardiac glycosides.

(k)Spot Test for Fixed Oils

Two milliliters of the extract were pressed between layers of Whatman filter paper for 2-3 minutes. The appearance of an oily spot on the paper indicated the presence of fixed oils.

Pharmacological Evaluation

2.3 Animals

Wistar rats (120–200 g) of similar age were obtained from an authenticated breeder. They were housed in polypropylene cages at 25±2°C with 43–57% humidity and a 12-hour light/dark cycle. Rats were fed a standard pellet diet, with food withdrawn 24 hours before the experiment but water allowed. The experimental procedures were approved by IAEC (Reg. No. UIP/IAEC/Feb.-2024/01).

2.4 Anti-Peptic Ulcer Activity

2.4.1. Pylorus Ligation-Induced Peptic Ulcer Model

Rats were acclimatized to the experimental lab a day prior and fasted overnight. Group I and II received oral saline, Group III received Ranitidine (20 mg/kg p.o.), and Groups IV and V were treated with the test extract ANLE at 250 mg/kg and 500 mg/kg p.o., respectively, 1 hour before the experiment.

The rats were anesthetized with Ketamine (60 mg/kg, i.p.), and a midline incision exposed the stomach for pylorus ligation. The incision was sutured, and animals were allowed to recover. After 4 hours, animals were sacrificed, and the stomachs were excised. Ulcers were examined under 10x magnification and scored based on severity. Gastric contents were centrifuged, and acidity was measured by titration using 0.01 N NaOH.

2.4.2. Histopathological Study

The stomach were exposed and dissected vertically after sacrificing the animals. The tissues were stored in airtight containers with formalin at a temperature below 37°C for histopathological evaluation. The stomach lesions were examined under a light microscope. Histopathological analysis was conducted at United Diagnostic & Research, Civil Lines, Prayagraj.

3. Determination of Biochemical Parameters

3.1. Volume of Gastric Acid

The stomach contents were collected and centrifuged at 1000 rpm for 10 minutes, and the volume of the supernatant was measured .

3.2. pH Measurement

One milliliter of the supernatant was diluted with 10 ml of distilled water, and the pH was measured using a pH meter .

3.3. Determination of Free Acidity (FA) and Total Acidity (TA)

For free acidity determination, 1–3 drops of methyl orange indicator were added to 1 ml of the supernatant, and the mixture was titrated with 0.01 N NaOH until a yellow-orange color developed. The volume of NaOH used indicated free acidity. Total acidity was determined by adding phenolphthalein indicator to 1 ml of the supernatant, followed by titration until a pink color appeared. The total volume of NaOH used indicated total acidity .

4. Statistical Analysis

Data were analyzed using one-way ANOVA followed by Sidak's multiple comparison test, performed using GraphPad Prism 10 software .

5. Results

5.1. Physiochemical parameters

The analytical evaluation of the plant material revealed several key parameters. The content of foreign matter was 0%. The loss on drying (LOD) at 105°C was 1.2%. The alcohol-soluble extractive value was 6.25%, while the water-soluble extractive value was slightly higher at 6.75%. The total ash value was recorded as 5.5%, with the acid-insoluble ash value at 4.5%. Additionally, the water-soluble ash value was found to be 2.75% (table 1).

Table 1: Physiochemical parameters of *Acacia nilotica* leaves powder drug

S. No.	Parameter	Value (%)
1.	Foreign matter	0%
2.	LOD at 105°C	1.2%
3.	Alcohol soluble extractive value	6.25%

4.	Water soluble extractive value	6.75%
5.	Total ash value	5.5%
6.	Acid insoluble ash value	4.5%
7.	Water soluble ash value	2.75%

5.2. Phytochemical Analysis

The phytochemical screening of the plant extract revealed the presence of several constituents. Alkaloids were identified using Tannic acid, Hager's, Dragendorff's, and Wagner's tests, with all tests showing positive results. For steroids, the Libermann-Burchard and Salkowski tests yielded positive results, while the Sulphur test was negative. Saponins were confirmed by a positive result in the Foam test. Carbohydrates were detected using Molisch's, Fehling, and Benedict's tests, all of which returned positive results. Glycosides were identified through positive Keller-Killiani and Legal's tests. Proteins were detected using Biurett's, Xanthoproteic, and Millon's tests, all of which were positive. Flavonoids were confirmed by positive Shinoda and Sulphuric tests. Lastly, tannins were detected through positive results in Bromine water and Ferric Chloride tests, but the Lead acetate test was negative (Table 2).

Table 2: Phytochemical analysis of extract of *Acacia nilotica* leaves

S. No.	Constituents	Test	Results
1.	Alkaloids	• Tannic acid test • Hager's test • Dragendorff's test • Wagner's test	+ + + +
2.	Steroids test	• Libermann-Burchard test • Sulphur test • Salkowski test	+ - +
3.	Saponins	• Foam test	+
4.	Carbohydrates	• Molisch's test • Fehling test • Benedict's test	+ + +
5.	Glycoside	• Keller killiani test • Legal's test	+ +
6.	Proteins	• Biurett's test • Xanthoproteic test • Millon's test	+ + +

7.	Flavanoids	• Shinoda test • Sulphuric test	+ +
8.	Tannins	• Bromine water test • Ferric Chloride test • Lead acetate test	+ + -

(+) present, (-) absent

5.3 Anti-Peptic Ulcer Activity

Administration of *Acacia seyal* leaf extract (ASLE) orally resulted in a significant decrease in the ulcer index. Both the high dose (500 mg/kg p.o.) and the low dose (250 mg/kg p.o.) of ASLE were effective in reducing the ulcer index. The reduction in ulcer levels observed with the standard drug, Ranitidine (20 mg/kg p.o.), was more pronounced and statistically significant ($P<0.0001$) compared to the effects of ASLE (Table 3).

Table 3: Effects of hydroalcoholic extract of *Acacia nilotica* leaves in Pylorus ligation induced peptic ulcer in rats.

Groups	**UI (Ulcer Index)**	**GV (Gastric Volume) (ml/100 g)**	**FA (Free Acidity) (mEq/1/100 g)**	**TA (Total acidity) (mEq/1/100 g)**	**pH**
Negative control	1.00±0.40	1.46±0.22	12.25±0.85	21.10±0.44	3.61±0.03
Positive control	4.31±0.19^{p}	2.91±0.29^{p}	55.53±0.94^{p}	101.86±0.85^{p}	1.66±0.23^{p}
Ranitidine 20 mg/kg	2.00±0.50^{p}	1.61±0.44^{p}	19.33±0.36^{p}	46.56±0.77^{p}	2.91±0.01^{p}
ASLE 250 mg/kg	2.41±0.22^{p}	1.95±0.29^{r}	53.06±0.65^{p}	99.03±0.60^{p}	2.20±0.10^{p}
ASLE 500 mg/kg	2.18±0.56^{p}	1.76±0.44^{r}	33.83±0.65^{p}	67.05±0.63^{p}	2.63±0.03^{p}

The values in each group are represented as mean±SD, with n = 6. The positive group was compared to the negative control group using Sidka's multiple comparison test& the drug-treated groups were compared to the positive control group after that. Where, $P<0.0001^{****}$,$P<0.001^{***}$, $P<0.01^{**}$, $P<0.05^{*}$, $P>0.05$.

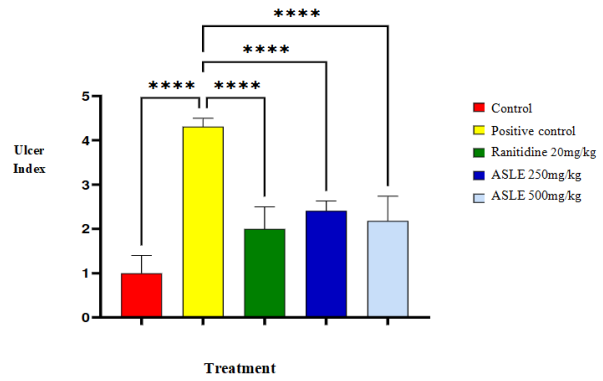


Fig.1 Ulcer Index of different treatment groups

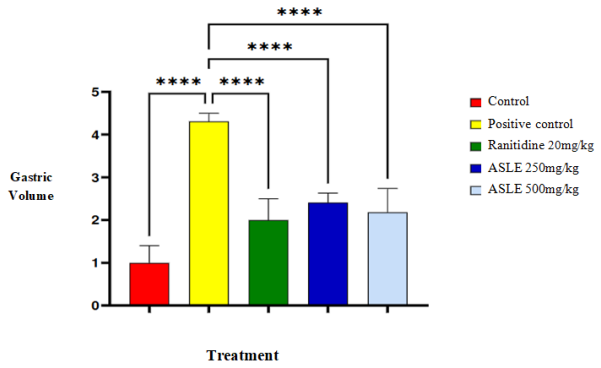


Fig:2 Gastric volume (GV) of different treatments

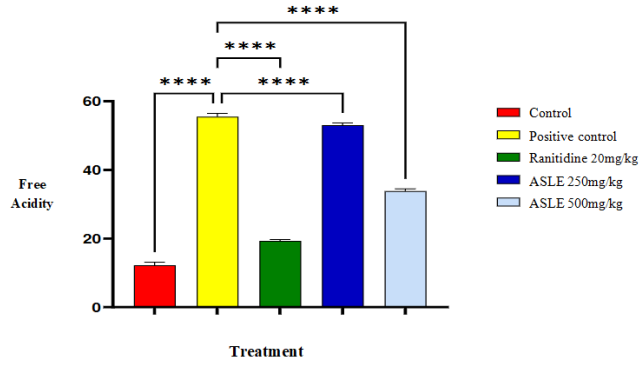


Fig.3 Free Acidity of different treatment groups

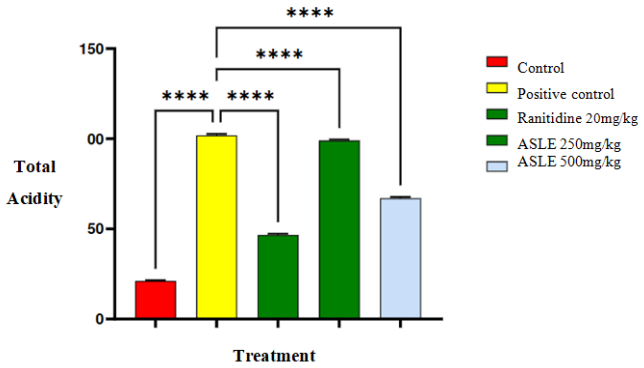


Fig.4 Total Acidity of different treatment groups

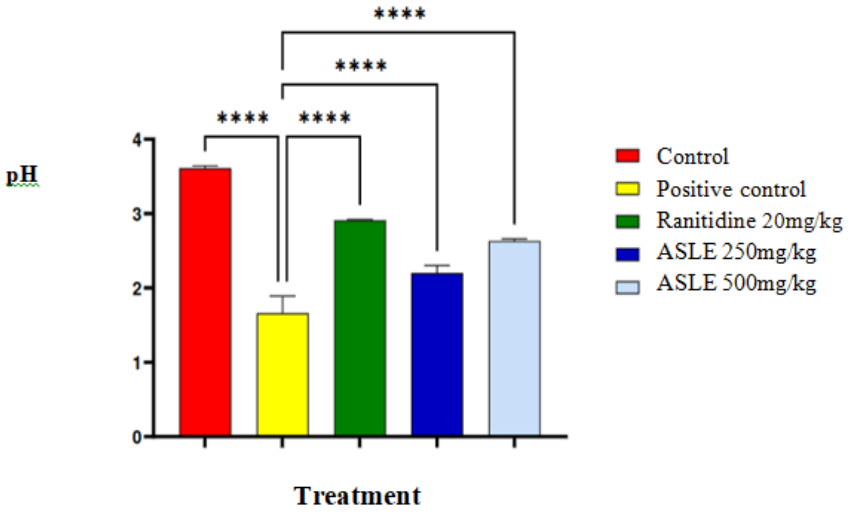
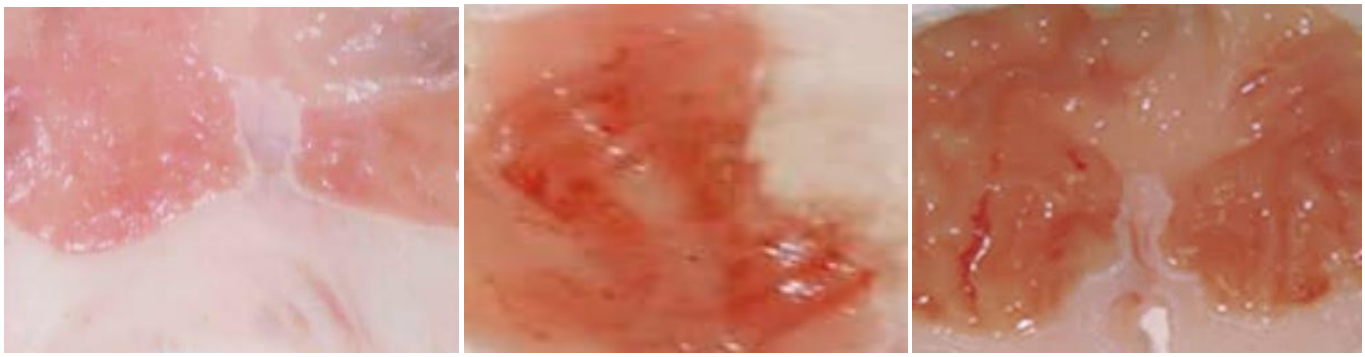


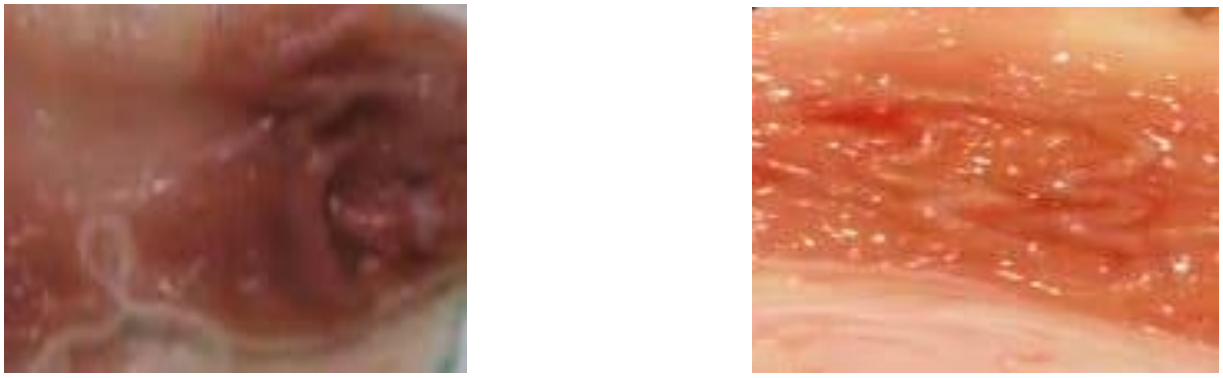
Fig.3 Gastric pH of different treatment groups

5.4 histopathological Study

Histopathological examination of the stomach from the normal control group (Fig. A) revealed minimal ulceration with only small gastric lesions, and the cell arrangement was uniformly maintained throughout the greater curvature of the stomach. In contrast, rats in the positive control group (Fig. B) exhibited severe cellular deformation due to ulceration. Rats treated with Ranitidine (20 mg/kg p.o.; Fig. C) displayed well-organized cell structures. Similarly, rats administered the test drug (ASLE 250 mg/kg p.o.; Fig D) showed reduced ulceration compared to the positive control group. Moreover, treatment with a higher dose of the test drug (ASLE 500 mg/kg p.o.; Fig. E) resulted in a significantly lower degree of ulceration compared to the positive control group (Fig.6,7).

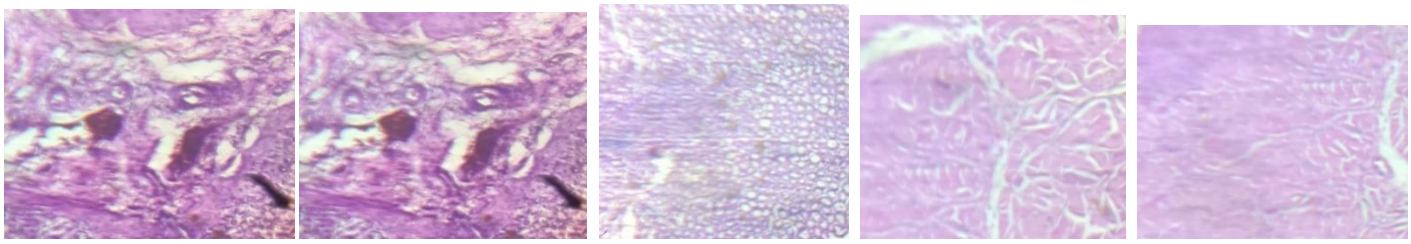


(B) Negative control **(C)** positive control **(A)** Ranitidine treated



(D) Extract (ASLE) treated **(E)** Extract (ASLE) treated

Fig.6 The stomach of the pylorus-ligated rat was dissected & the rumen of the stomach where the ulcers were predominantly found & seen the preventive effect of drugs



(A) (B) (C) (D) (E)

(A) Negative control, **(B)**Positive control, **(C)** Ranitidine 20 mg/kg p.o., **(D)** ASLE 250 mg/kg p.o., **(E)** ASLE500 mg/kg p.o.

Fig.7 Histological sections of the stomach of rats

Discussion

The study demonstrated the anti-ulcer potential of *Acacia seyal* leaf extract (ASLE) using a pylorus ligation-induced ulcer model in Wistar rats. Histopathological analysis revealed that ulceration was significantly mitigated in the groups treated with ASLE, both at low (250 mg/kg p.o.) and high (500 mg/kg p.o.) doses, as compared to the positive control group. The reduction in ulcer formation was particularly pronounced at the higher dosage, suggesting a dose-dependent effect. Furthermore, the standard drug, Ranitidine (20 mg/kg p.o.), showed the most significant reduction in ulcer index, with organized cellular architecture in the gastric mucosa, highlighting its potent anti-ulcer activity.

The decrease in ulcer index in the ASLE-treated groups suggests that the extract possesses gastroprotective properties, potentially due to its phytoconstituents such as alkaloids, saponins, flavonoids, and tannins, which have been reported in earlier studies to exert antioxidant, anti-inflammatory, and mucosal protective effects. These bioactive compounds may inhibit the secretion of gastric acid, enhance mucosal defense mechanisms, or neutralize free radicals, thereby reducing ulcer formation. The findings also align with previous research that supports the traditional use of *Acacia nilotica* in treating gastric disorders.

Conclusion

The present study concludes that *Acacia seyal* leaf extract exhibits significant anti-ulcer activity, as evidenced by the reduction in ulcer index and the improvement in gastric histopathology in treated rats. The effect is dose-dependent, with the higher dose (500 mg/kg p.o.) showing greater efficacy. While Ranitidine remains a potent anti-ulcer agent, the results suggest that *Acacia seyal* may offer a promising natural alternative for managing peptic ulcers, though further studies are required to fully elucidate its mechanism of action and clinical potential.

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