INFORMATICS JOURNALS

Research Article

Journal of Natural Remedies
DOI: 10.18311/jnr/2026/55230

eISSN: 2320-3358 | Vol 26(7), 2026 | pp. 1821-1830

Evaluation of Efficacy and Safety of Ethanolic Extract of *Prosopis juliflora* Seeds in NSAID-induced Gastric Ulcer Model in Wistar Albino Rats

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Abstract

Background: Peptic ulcer disease remains a major global health concern, with nonsteroidal anti-inflammatory drugs (NSAIDs), particularly aspirin, being a leading cause of gastric mucosal injury. Aspirin induces ulceration through irreversible cyclooxygenase-1 inhibition, depletion of protective prostaglandins, increased gastric acid secretion, and oxidative stress. Although proton pump inhibitors are effective, their long-term use may cause adverse effects, highlighting the need for safer phytotherapeutic alternatives. *Prosopis juliflora*, rich in flavonoids, tannins, alkaloids, and phenolic compounds, has shown gastroprotective potential. **Aim:** To evaluate the antiulcer activity of the ethanolic extract of *P. juliflora* seeds against aspirin-induced gastric ulceration in Wistar albino rats. **Methods:** Wistar albino rats were randomly divided into five groups: normal control (normal saline, 1 mL/kg), disease control (aspirin, 150 mg/kg), standard (omeprazole, 20 mg/kg), and *P. juliflora* extract-treated groups (250 and 500 mg/kg). Gastric ulceration was induced by oral aspirin administration one hour after pretreatment. Six hours later, gastric tissues were examined for ulcer index, ulcer severity, percentage ulcer inhibition, gastric juice volume, pH, free acidity, total acidity, and gross gastric mucosal changes. **Results:** The disease control group showed severe gastric injury with a high ulcer index (2.49 ± 0.35), increased gastric juice volume (5.16 ± 0.52 mL), elevated free acidity (33 ± 4.8) and total acidity (50.8 ± 5.49), and reduced gastric pH (2.2 ± 0.45). Pretreatment with *P. juliflora* significantly reduced ulceration in a dose-dependent manner, producing ulcer inhibition of 53.41% and 55.80% at 250 and 500 mg/kg, respectively ($p < 0.01$). Omeprazole produced 70.28% ulcer inhibition. **Conclusion:** The ethanolic extract of *P. juliflora* seeds demonstrated significant gastroprotective and antiulcer activity, supporting its potential as a phytotherapeutic candidate for the prevention and management of NSAID-induced peptic ulcer disease.

Major Findings: Ethanolic extract of *Prosopis juliflora* seeds significantly reduced aspirin-induced gastric ulceration, decreased gastric acidity, and increased gastric pH. These findings demonstrate its significant gastroprotective activity against NSAID-induced gastric mucosal damage.

Keywords: Antiulcer Activity, Aspirin-induced Ulcer, Flavonoids, Gastroprotection, Peptic Ulcer Disease, *Prosopis juliflora*, Tannins, Wistar Albino Rats

(Article Chronicle: Article Received on: 06.03.2026; Revised on: 05.06.2026; Accepted on: 12.06.2026)

1. Introduction

Peptic Ulcer Disease (PUD) is characterised by an erosion in the mucosal lining of the stomach and duodenum extending deep into the muscularis propria. PUD is a multifactorial condition associated with an imbalance between defensive (mucus-bicarbonate barrier, mucosal blood flow, and prostaglandin-mediated defence) and aggressive factors (acid, pepsin, *H. pylori*, and NSAIDs). *Helicobacter pylori* infection is the principal etiological factor, accounting for 70-90 % of duodenal ulcers and 50-70% of gastric ulcers. and NSAID use are the major contributing factors to the development of peptic ulcers[1]. Cigarette smoking

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impairs ulcer healing, and alcohol consumption potentiates the risk of mucosal injury and bleeding[2]. The main complications of peptic ulcer disease include bleeding, perforation, and gastric outlet obstruction. According to data from the 2019 Global Burden of Disease study, peptic ulcer disease affected roughly 32.9 million individuals worldwide and was responsible for approximately 817,000 deaths[3].

Non-steroidal anti-inflammatory drugs, such as aspirin and indomethacin, are the most frequently prescribed over-the-counter medications worldwide and are widely used for their analgesic, antipyretic, and anti-inflammatory properties. Despite their widespread therapeutic benefits in managing pain and inflammatory conditions, NSAIDs are strongly associated with significant gastrointestinal (GI) toxicity, particularly the development of peptic ulcers and life-threatening complications, including bleeding, perforation, and obstruction[4].

Aspirin-induced peptic ulceration results from a combination of topical mucosal injury caused by its direct irritant effect and systemic prostaglandin suppression. Aspirin-induced irreversible COX-1 inhibition decreases the synthesis of protective prostaglandins, which play an important role in mucus and bicarbonate production, increases mucosal blood flow, and inhibits gastric acid production[5]. COX inhibition leads to a deficiency of prostaglandins, thereby affecting gastric mucosal integrity. Secondly, aspirin is a weakly acidic drug that remains non-ionised in the acidic gastric lumen, enabling it to diffuse across the phospholipid membranes of surface epithelial cells. The neutral intracellular pH of the epithelial cells causes aspirin to become ionized and get trapped inside the intestinal epithelium (ion trapping), leading to uncoupling of mitochondrial oxidative phosphorylation, leakage of Ca^{2+} from mitochondria, increased production of reactive oxygen species, and imbalance in the sodium-potassium ratio with cellular osmotic imbalance and dysfunction of tight intracellular junctions and an increase in permeability of the superficial intestinal epithelium, leading to invasion of aggressive luminal contents such as bile acid, hydrolytic and proteolytic enzymes, and invasion of intestinal bacteria, resulting in neutrophil chemotaxis causing non-specific inflammation, ulcers, perforations, bleeding, and strictures[6,7].

Current pharmacological management of PUD primarily relies on Proton Pump Inhibitors (PPIs), H_2-receptor antagonists, antacids, and *H. pylori* eradication regimens. While these agents are clinically effective, prolonged use is associated with several adverse effects, including hypomagnesemia, *Clostridioides difficile* infection, increased susceptibility to enteric infections, and potential risk of gastric carcinoid tumours with long-term PPI use[8,9]. Furthermore, growing concerns regarding antibiotic resistance in *H. pylori* eradication therapy have necessitated the search for alternative therapeutic strategies[10]. In this context, medicinal plants have garnered increasing scientific attention as potential sources of novel gastroprotective agents with favourable safety profiles.

Prosopis juliflora, commonly referred to as mesquite, is widespread in the semi-arid and arid places worldwide. It is a drought-resistant leguminous plant belonging to the family Fabaceae, subfamily Mimosoideae, with 44 species worldwide[11]. *P. juliflora* is native of Central and South America. In India, the species has adapted across Tamil Nadu, Andhra Pradesh, Rajasthan, Madhya Pradesh, Karnataka, Haryana, Maharashtra, Odisha, and Uttar Pradesh[12]. *P. juliflora* is a fast-growing perennial tree that can reach heights of 10–15 meters. It possesses bipinnate leaves, greenish-yellow cylindrical flowers, curved, elongated seed pods, and sharp axillary thorns. *P. juliflora* contains a wide array of bioactive compounds, including alkaloids (notably juliflorine and julifloricine), flavonoids, saponins, tannins, and phenolic acids[13]. These phytoconstituents account for its antiulcer, anticancer, antioxidant, antipyretic, anthelmintic, cytotoxicity, antigiardial, amoebicidal, anti-inflammatory, antidiabetic and analgesic activities[14-19]. Traditional medicine utilised different parts of this plant—roots, leaves, pods and flowers—for treating a variety of ailments, including gastrointestinal disorders, skin infections, conjunctivitis, toothache, and wound healing[20].

Flavonoids and tannins present in *P. juliflora seeds* are known to strengthen the gastric mucosal barrier by stimulating mucus secretion, inhibiting acid secretion, and scavenging reactive oxygen species[21]. Alkaloids such as juliflorine have demonstrated notable anti-inflammatory activity through inhibition of pro-inflammatory cytokines, including TNF-α and IL-6[22]. Phenolic acids exert cytoprotective effects

through modulation of oxidative stress pathways and maintenance of mitochondrial membrane integrity[23]. Hence, the present study was designed to evaluate the gastroprotective and antiulcer activity of the ethanolic extract of *Prosopis juliflora* seeds against aspirin-induced peptic ulcer in a rat model.

2. Materials and Methods

2.1 Ethics Approval and Animal Care

The study was initiated following approval by the Institutional Animal Ethics Committee (IAEC) of Chettinad Hospital and Research Institute, Kelambakkam. (IAEC approval No. IAEC3/Propoal:8/A.Lr.63/Dt;22/8/2022), and conducted in strict accordance with the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA), Government of India. The study adhered to the ARRIVE 2.0 reporting guidelines for *in vivo* animal research. A total of 30 adult male Wistar albino rats (180–220 g, 8–10 weeks old) were obtained from Biogen Laboratory animal facility, Reg. No.971/PO/RcBiBt/S/2006/CCSEA, Bangalore and housed in standard polypropylene cages (five animals per cage) under controlled conditions (temperature 22 ± 2 °C, relative humidity 50–60 %, 12 h light/dark cycle). Standard pelleted diet and filtered water were provided *ad libitum* throughout the acclimatisation period of seven days. Rats were fasted for 36 h before drug administration with free access to water.

2.2 Plant Material and Preparation of Extract

Seeds of *Prosopis juliflora* (Sw.) DC. (*Fabaceae*) were collected near Madipakkam, Chennai, Tamil Nadu. The plant and the seeds were identified and authenticated by an authorised botanist before preparation of the extract. Seeds were shade-dried at room temperature, coarsely powdered, and subjected to successive solvent extraction using a Soxhlet apparatus. 50 g of seed was macerated in 250 ml of ethanol at room temperature with occasional shaking for 24 hr. The extract was filtered through Whatman No. 1 filter paper, concentrated under reduced pressure using a rotary evaporator at 55°C, and lyophilised to yield 5.05 g of extract. The extract was stored at −20°C in airtight containers until use. For administration, the extract was freshly suspended in 0.5% w/v carboxymethylcellulose (CMC) sodium solution on each experimental day.

2.4 Drugs and Chemicals

Aspirin (acetylsalicylic acid) and omeprazole were of pharmaceutical grade and procured from a local licensed pharmaceutical supplier, Chennai, India. Sodium hydroxide (0.01 N), Topfer's reagent (dimethylaminoazobenzene), phenolphthalein indicator, and carboxymethylcellulose sodium were of analytical grade and obtained from HiMedia Laboratories Pvt. Ltd., Chennai, India. All reagents were prepared fresh on the day of the experiment.

2.5 Study Design and Grouping

Following acclimatisation, rats were randomly allocated into five groups, each with five rats. All rats were fasted for 36 hr before aspirin administration, with free access to water.

Group I (Normal Control): Normal saline (1 mL/kg, p.o.).

Group II (Disease Control): Aspirin (150 mg/kg, p.o.).

Group III (Standard Treatment): Omeprazole (20 mg/kg, p.o.).

Group IV (*P. juliflora* low Dose): *Prosopis juliflora* seed extract (250 mg/kg, p.o.).

Group V (*P. juliflora* high dose): *Prosopis juliflora* seed extract (500 mg/kg, p.o.).

2.6 Induction of Ulcer

Gastric ulcers were induced according to the method of Kulkarni (2002), with minor modifications[24]. Rats in Groups II–V received a single oral dose of aspirin (150 mg/kg, suspended in 0.5% CMC) via gavage, administered 1 hr after the respective test or standard drug treatment. Group I (normal control) received an equivalent volume of 0.5% CMC at corresponding time points. Animals were returned to their home cages without access to food but with free access to water for 6 h following aspirin administration.

Gastric ulcers were induced in all treatment groups (Groups II-V) by oral administration of aspirin at a dose of 150 mg/kg, suspended in 0.5% carboxymethyl cellulose, using an oral gavage. Aspirin (150 mg/kg) was administered 1 hour after the test and standard drugs, respectively[24].

2.7 Euthanasia and Stomach Excision

Six hours after aspirin administration, all animals were humanely euthanised by an overdose of Halothane (3-5 ml) in accordance with CCSEA guidelines. The abdomen was opened by midline laparotomy. The oesophagus and duodenum were ligated at the gastroesophageal junction and pylorus, respectively, to prevent loss of gastric contents. The intact stomach was carefully excised, and gastric juice was collected by puncturing the greater curvature and draining into a pre-weighed centrifuge tube. The stomach was then opened along the greater curvature and rinsed gently with cold normal saline. The gastric mucosa was examined macroscopically under a magnifying lens, and all samples were processed immediately.

2.8 Study Assessments

2.8.1 Macroscopic Ulcer Scoring and Ulcer Index

The gastric mucosa was evaluated macroscopically for haemorrhage, congestion, oedema, and mucosal erosions by two independent observers blinded to group allocation. Ulcers were graded using a validated scoring scale[25], as detailed in Table 1.

The ulcer index (UI) for each group was calculated using the formula:

$$UI = (UN + US + UP) \times 10^{-1}$$

where UN is the mean number of ulcers per rat, US is the mean severity score per rat, and UP is the percentage of rats with ulcers in each group[25].

The percentage of ulcer protection conferred by each treatment was calculated as:

$$\% \text{ Protection} = [(UI_a - UI_t) / UI_a] \times 100$$

where UI_a is the ulcer index of the disease control group (Group II), and UI_t is the ulcer index of the treatment group under evaluation.

Table 1. Macroscopic ulcer scoring scale for gastric mucosal evaluation

Score	Macroscopic appearance
0	Normal gastric mucosa; no visible lesions
0.5	Red colouration of the mucosa
1.0	Spot ulcers (punctate haemorrhages, ≤1 mm)
1.5	Hemorrhagic streaks (>1 mm linear lesions)
2.0	Deep ulcers (penetrating the mucosa)

2.8.2 Determination of Gastric Secretory Parameters

The collected gastric juice was centrifuged at 1,000 × g for 10 min at 4°C to remove mucus and debris. The following parameters were measured from the clarified supernatant.

2.8.3 Gastric Juice Volume

The volume of gastric juice was measured by transferring the supernatant to a graduated glass measuring cylinder and expressed in millilitres (mL).

2.8.4 Gastric pH

The pH of the gastric juice was measured immediately after collection using a calibrated digital pH meter, standardised with buffer solutions before each measurement session. The electrode was immersed in the gastric juice, and the stable reading was recorded.

2.8.5 Determination of Free Acidity

A 1.0 mL aliquot of gastric juice was transferred to a 100 mL conical flask and diluted with 10 mL of distilled water. Two to three drops of Topfer's reagent (0.5% w/v dimethylaminoazobenzene in ethanol) were added, and the solution was titrated against 0.01 N sodium hydroxide (NaOH) until the red colour changed permanently to yellowish-orange, marking the endpoint. Free acidity was expressed in milliequivalents per litre (mEq/L) using the formula[25]:

$$\textit{Acidity (mEq/L)} = \textit{(Volume of NaOH consumed (mL)} \times \textit{Normality of NaOH} \times 1000) / \textit{Volume of gastric juice taken (mL)}$$

2.8.6 Determination of Total Acidity

Following the free acidity endpoint, two to three drops of phenolphthalein indicator (1% w/v in 70% ethanol) were added to the same flask, and titration continued with 0.01 N NaOH until a persistent pale-pink colour appeared. The total volume of NaOH consumed from the beginning of the titration to this final endpoint was used to calculate total acidity using the same formula stated above[25].

2.9 Post-experimental Procedures

Upon completion of the experimental protocol, animal carcasses were disposed of in accordance

with institutional biosafety regulations and CCSEA guidelines. Disposal was carried out by incineration using a G.J. Multiclave system.

2.10 Statistical Analysis

The sample size was calculated using the formula: n=DF/k+1.

where DF denotes the degrees of freedom and *k* represents the number of experimental groups. Based on a calculated degrees of freedom value of 20 and five experimental groups ($k = 5$), a sample size of five rats per group was determined and used for the study.

All data are expressed as mean ± Standard Error of the Mean (SEM). The normality of data distribution was assessed using the Shapiro–Wilk test. Intergroup comparisons were performed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test. Statistical analyses were performed using GraphPad Prism (version 9.0). A p-value of <0.05 was considered statistically significant.

3. Results

3.1 Ulcer Scoring

Results showed that administration of aspirin (150 mg/kg) produced significant gastric mucosal damage as evidenced by an increase in mean ulcer score (12.4 ± 1.51), mean ulcer severity score (11.5 ± 2.17), and ulcer index (2.49 ± 0.35) when compared to the vehicle-treated control group, which indicated the ulcerogenic effect of aspirin. Pretreatment with the standard drug (T. Omeprazole 20 mg/kg) markedly reduced aspirin-induced gastric lesions. The standard-treated group demonstrated a substantial decrease in the mean ulcer score (3.2 ± 1.30), mean ulcer severity score (3.8 ± 1.71), and ulcer index (0.74 ± 0.24), corresponding to a 70.28% inhibition of ulceration relative to the disease control, as shown in Table 2.

Administration of *Prosopis juliflora* seed extract produced a dose-dependent gastroprotective effect. At 250 mg/kg, *P. juliflora* significantly reduced the mean ulcer score (4.8 ± 0.83), mean ulcer severity score (5.9 ± 1.02), and ulcer index (1.16 ± 0.15), resulting in 53.41% ulcer inhibition compared to the disease control group. A dose-dependent, significant reduction in ulcer parameters was observed at 500 mg/kg, with mean ulcer score, mean ulcer severity score, and ulcer index values of 4.0 ± 0.70, 6.2 ± 1.15, and 1.10 ± 0.18, respectively, corresponding to a 55.8% inhibition of ulceration, as shown in Figure 1.

Overall, *P. juliflora* extract exhibited significant gastroprotection against aspirin-induced gastric ulcers, with the high dose of *P. juliflora* (500 mg/kg) showing slightly greater efficacy. However, the gastroprotective effect of *P. juliflora* at both doses (250 mg/kg and 500 mg/kg) was comparatively lower than that observed with the standard antiulcer drug.

3.2 Gastric Secretory Parameters

In the aspirin-induced gastric ulcer model, the disease control group exhibited a significant increase in gastric secretory parameters compared to the normal control group (Table 2). The volume of gastric juice was markedly elevated (5.16 ± 0.52 mL vs. 3.9 ± 0.46 mL), accompanied by a significant reduction in gastric pH (2.2 ± 0.45 vs. 3.26 ± 0.55). Correspondingly, free acidity and total acidity were significantly increased in the diseased control group (33 ± 4.8 and 50.8 ± 5.49, respectively) compared to the normal control rats, as shown in Table 3.

Table 2. Effect of *Prosopis juliflora* on gastric ulcer severity

Groups	Total Ulcer score	Total Ulcer Severity Score	Ulcer index	% Ulcer Inhibition
Control	0 ± 0	0 ± 0	0 ± 0	100
Disease Control	12.4 ± 1.51	11.5 ± 2.17	2.49 ± 0.35	-
Standard	3.2 ± 1.30**	3.4 ± 1.71**	0.74 ± 0.24**	70.28
P.juliflora 250 mg	4.8 ± 0.83**	5.9 ± 1.02**	1.16 ± 0.15**	53.41
P.juliflora 500 mg	4 ± 0.70**	6.2 ± 1.15**	1.1 ± 0.18**	55.8

Values are expressed as Mean±S.D, n=5, *p<0.05, **p<0.01, as compared with disease control using one-way ANOVA followed by Post hoc Tukey's test.

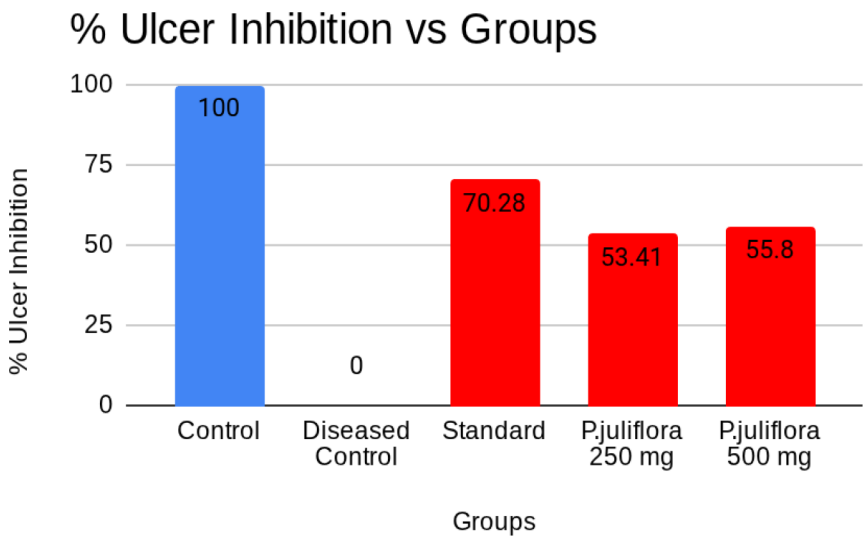


Figure 1. Percentage of ulcer inhibition.

Table 3. Gastric secretory parameters in aspirin-induced ulceration

Groups	Volume of Gastric Juice	PH	Free Acidity	Total Acidity
Control	3.9 ± 0.46	3.26 ± 0.55	17 ± 4.3	27.2 ± 5.5
Disease Control	5.16 ± 0.52	2.2±0.45	33 ± 4.8	50.8 ± 5.49
Standard	2.19 ± 0.52 **	5.04 ± 0.57 **	19.2 ± 3.49 **	29.4 ± 5.59 **
P.juliflora 250 mg	3.45 ± 0.34 **	3.66 ± 0.33 **	24.4 ± 3.97 *	40.8 ± 4.7 *
P.juliflora 500 mg	4.06 ± 0.37 **	4.12 ± 0.25 **	19.8 ± 1.3 **	35.4 ± 4.82 **

Values are expressed as Mean±S.D, n=5, *p<0.05, **p<0.01, as compared with disease control using one-way ANOVA followed by Post hoc Tukey's test. Treatment with the standard drug (T. Omeprazole 20 mg/kg) resulted in a significant reduction in gastric juice volume (2.19 ± 0.52 mL, **p < 0.01) and a significant increase in gastric pH (5.04 ± 0.57, **p < 0.01) when compared to the ulcer control group. Free acidity (19.2 ± 3.49) and total acidity (29.4 ± 5.59) were significantly reduced with **p < 0.01. (Table 3)

Administration of *Prosopis juliflora* extract at a dose of 250 mg/kg produced a significant decrease in gastric juice volume (3.45 ± 0.34, **p < 0.01) and a significant increase in gastric pH (3.66 ± 0.33, **p < 0.01) compared to the ulcer control group. This dose also resulted in significant reductions in free acidity (24.4 ± 3.97) and total acidity (40.8 ± 4.7) (p < 0.05), as shown in Table 3.

High doses of *P. juliflora* seed extract (500 mg/kg) demonstrated a more pronounced gastroprotective effect, as evidenced by a significant increase in gastric pH (4.12 ± 0.25, **p < 0.01) and a significant reduction in free acidity (19.8 ± 1.3, **p < 0.01) and total acidity (35.4 ± 4.82, **p < 0.01) when compared to the disease control group. The observed effects at this dose were comparable to those produced by the standard treatment (Table 3).

3.3 Gross Examination of Gastric Mucosa

The normal control group exhibited a smooth, intact mucosal surface without any visible lesions. In contrast, the aspirin-treated diseased control group demonstrated severe gastric mucosal damage characterised by multiple hemorrhagic streaks, extensive mucosal erosions, ulcerative lesions, and marked congestion. Rats treated with the standard drug showed substantial preservation of mucosal integrity with a marked reduction in the number and severity of hemorrhagic and ulcerative lesions. Treatment with *Prosopis juliflora* seed extract at doses of 250 and 500 mg/kg resulted in a dose-dependent attenuation of aspirin-induced gastric erosions, evidenced by fewer ulcerative lesions, reduced hemorrhagic streaks, and improved mucosal integrity, with the high dose exhibiting near-normal gross morphology as shown in Figure 2.

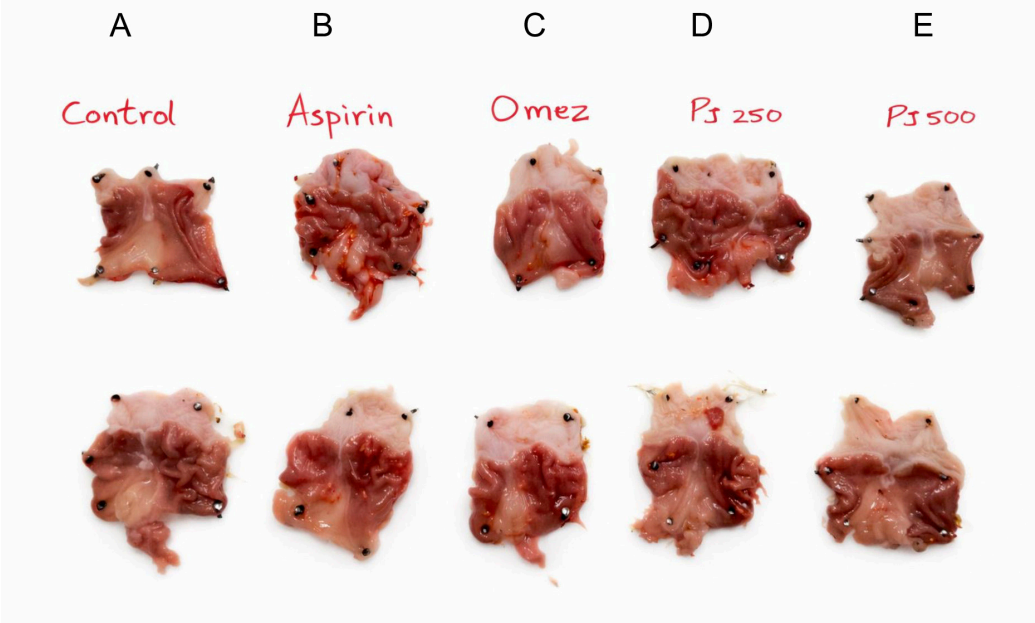


Figure 2. Gross examination of the gastric mucosa. **(A).** Normal control showing intact gastric mucosa; **(B).** Aspirin-treated diseased control showing severe hemorrhagic streaks and ulcerative lesions; **(C).** Standard drug-treated group showing significant reduction in gastric lesions with preservation of mucosal integrity; **(D).** *Prosopis juliflora* seed extract (250 mg/kg) showing reduced gastric lesions; **(E).** *Prosopis juliflora* seed extract (500 mg/kg) showing near-normal mucosal appearance.

4. Discussion

Peptic Ulcer Disease (PUD) continues to represent a major global health concern, particularly in populations with high exposure to Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). Epidemiological evidence indicates substantial global morbidity and mortality associated with ulcer complications, especially bleeding and perforation[26]. Among NSAIDs, aspirin is extensively prescribed for its analgesic, anti-inflammatory, and cardioprotective effects; however, its potential to induce gastric mucosal injury remains clinically significant[27]. The present study demonstrated that the ethanolic extract of *Prosopis juliflora* seeds exerts significant gastroprotective effects against aspirin-induced gastric ulceration in Wistar albino rats.

Aspirin-induced ulcers primarily result from irreversible inhibition of cyclooxygenase enzymes, particularly COX-1, which is responsible for the synthesis of cytoprotective prostaglandins such as PGE2 and PGI2[28]. Prostaglandins play a crucial role in maintaining the integrity of the gastric mucosal barrier by stimulating the secretion of mucus and bicarbonate, enhancing mucosal blood flow, and promoting cellular regeneration[29]. The reduction in prostaglandin levels leads to a compromised mucosal defence, making the gastric mucosa more susceptible to the corrosive effects of hydrochloric acid and pepsin. In our study, the disease control group exhibited a high ulcer index (2.49 ± 0.35), confirming the successful induction of gastric mucosal damage by aspirin.

The present study aimed to evaluate the gastroprotective potential of *Prosopis juliflora* seed extract against aspirin-induced gastric ulcers in Wistar rats. Our findings demonstrate that *P. juliflora* significantly mitigates gastric mucosal damage, as evidenced by a substantial reduction in the total ulcer score, ulcer severity score, and ulcer index. *P. juliflora* (500 mg/kg) exhibited a 55.8% ulcer inhibition, while the 250 mg/kg dose showed 53.41% inhibition, both of which were highly significant ($p < 0.01$) compared to the disease control. These results suggest that *P. juliflora* possesses potent anti-ulcer properties, likely mediated through a combination of cytoprotective, antioxidant, and anti-inflammatory mechanisms.

The Phytochemical analysis of *P. juliflora* has revealed the presence of flavonoids, tannins, alkaloids (like juliflorine), and phenolic compounds[30]. Flavonoids are well-known for their ability to enhance gastric mucosal defence by increasing prostaglandin

synthesis and scavenging reactive oxygen species[31]. Quercetin, in particular, has been shown to inhibit lipid peroxidation and increase the levels of endogenous antioxidants like superoxide dismutase and glutathione in the gastric mucosa[32]. These antioxidant properties are critical in counteracting the oxidative stress induced by aspirin, which generates free radicals that cause lipid peroxidation and subsequent mucosal necrosis.

Furthermore, the presence of tannins in *P. juliflora* may contribute to its gastroprotective effect through the formation of a protective protein-tannate complex over the gastric mucosa. This complex acts as a physical barrier, shielding the underlying gastric mucosa from the aggressive actions of acid and pepsin, thereby facilitating the healing process[33]. Tannins also possess astringent properties that can cause vasoconstriction, further reducing the severity of mucosal bleeding and inflammation.

Prosopis juliflora seed extract significantly reduced the Total Ulcer Severity Score in our treated groups (5.9 ± 1.02 and 6.2 ± 1.15 for 250 mg/kg and 500 mg/kg doses, respectively) compared to the disease control (11.5 ± 2.17), indicating its ability to preserve or restore the gastric mucosal barrier. This gastroprotective action is consistent with previous reports on other *Prosopis* species. For instance, *Prosopis farcta* has been shown to possess anti-ulcer activity in ethanol-induced models, attributed to its high content of tannins and flavonoids[34]. Similarly, *Prosopis africana* stem bark extract demonstrated protective effects against indomethacin-induced ulcers[35]. Our findings align with these studies, suggesting that the *Prosopis* genus is a rich source of bioactive compounds with significant gastroprotective potential.

In comparison with other medicinal plants, the anti-ulcer efficacy of *P. juliflora* (55.8% inhibition at 500 mg/kg) is comparable to that of *Amaranthus spinosus* (51.4% at 400 mg/kg) and *Acacia catechu* in similar aspirin-induced models[36,37]. While the standard drug in our study showed a higher inhibition rate (70.28%), the significant dose-dependent protection offered by *P. juliflora* highlights its potential as a natural alternative or adjunct therapy for managing NSAID-induced gastric disorders.

Long-term PPI therapy has been associated with adverse outcomes, including micronutrient malabsorption, renal dysfunction, and gut microbiome alterations, which can predispose individuals to infections[38]. This has fueled a growing interest in safer, long-term phytotherapeutic alternatives that can offer protection without the systemic liabilities of profound acid suppression.

The present findings suggest that *P. juliflora* seed extract may serve as a promising complementary anti-ulcer agent. While its efficacy was lower than omeprazole, it demonstrated substantial gastroprotection comparable to other established medicinal plant extracts.

5. Limitations

The study utilised a small sample size and an acute ulcer model. Future investigations should evaluate chronic ulcer models, oxidative stress markers (MDA, SOD, GSH), inflammatory mediators, and prostaglandin levels to elucidate further the mechanisms involved.

6. Conclusion

In conclusion, this study unequivocally demonstrates the significant gastroprotective efficacy of *Prosopis juliflora* seed extract against aspirin-induced gastric ulcers. The extract, administered at both 250 mg/kg and 500 mg/kg doses, effectively reduced ulcer formation and severity, highlighting its potent anti-ulcerogenic properties. These beneficial effects are likely attributable to the rich array of bioactive compounds, particularly flavonoids and tannins, which exert their actions through multiple mechanisms, including antioxidant defence, cytoprotection, and the reinforcement of the gastric mucosal barrier. The findings highlight the potential of *P. juliflora* as a valuable natural therapeutic agent for the prevention and management of NSAID-induced ulcers.

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Author Contribution

Conceptualisation- V.D.N. and A.R.; methodology- V.D.N. and A.R; validation- V.D.N. and A.R; formal analysis- V.D.N. and A.R; investigation- V.D.N, A.R. and S.D.; data curation- V.D.N. and A.R.; writing—original draft preparation- V.D.N. and A.R; writing—review and editing- V.D.N. and A.R; visualisation- V.D.N., S.D., and A.R.; supervision-A.R. All authors have read and agreed to the published version of the manuscript.