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Protective Role of *Pterospermum acerifolium* Ethyl Acetate Extract Against Aspirin-Induced Gastric Ulceration in Mice

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Abstract

Background: Gastric ulcer disease is a chronic ailment due to an imbalance between the aggressive factors present in gastric mucosal tissue and the protective ability of mastication, leading to the harm of mucous membrane and gastrointestinal destruction. These natural agents decrease gastric acid either by suppressing gastric acid secretion (acid suppressors), antacids, and cytoprotective agents (a kind of substance that protects cells from dying). Nevertheless, prolonged therapy with these agents is frequently associated with side effects and thus safer phytochemical approaches are urgently required. *Pterospermum acerifolium* has drawn attention because of its abundant phytochemical contents and documented antioxidative and anti-inflammatory activities. Aims: This study was designed to investigate antioxidant and anti-inflammatory properties of phytochemicals in *Pterospermum acerifolium* extract against aspirin-induced gastric ulceration in murine model. Results: Phytochemical screening (including HPLC, FTIR and LC-MS/MS) signalled the presence of multiple bioactive compounds in *P. acerifolium* extract. Histopathological examination revealed marked mucosal damage in ulcerated group as compared to omeprazole and *P. acerifolium* treated groups with preserved gastric mucosa integrity. Inflammatory cell infiltration and the architecture of the gastric mucosa were markedly improved after the administration of extract, and this protective effect may be attributed to its antioxidant and anti-inflammatory effects. Conclusions: The results show that *P. acerifolium* extract fraction has a good gastroprotection and may be candidate for potential therapeutic perspective.

Keywords: Antiulcer, *Pterospermum acerifolium*, Ethyl acetate, HPLC. Gallic acid

الدور الوقائي لمستخلص خلاص الإيثيل لنبات طبق العشاء ضد القرحة المعدية المستحثة بالأسبيرين
في الفئران

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الخلاصة

الخلفية: يُعدّ مرض قرحة المعدة مرضًا مزمنًا ناتجًا عن اختلال التوازن بين العوامل الضارة الموجودة في نسيج الغشاء المخاطي للمعدة وقدرة المضغ على الحماية، مما يؤدي إلى تلف الغشاء المخاطي وتدمير الجهاز الهضمي. تعمل هذه العوامل الطبيعية على تقليل حموضة المعدة إما عن طريق تثبيط إفراز حمض المعدة (مبطلات الحموضة)، أو مضادات الحموضة، أو العوامل الواقية للخلايا (وهي نوع من المواد التي تحمي الخلايا من الموت). ومع ذلك، غالبًا ما يرتبط العلاج المطول بهذه العوامل بآثار جانبية، وبالتالي هناك حاجة ماسة إلى مناهج علاجية نباتية أكثر أمانًا. وقد حظي نبات طبوق العشاء باهتمام كبير نظرًا لمحتواه الغني من المركبات الكيميائية النباتية وخصائصه المضادة للأكسدة والالتهابات الموثقة. الأهداف: صُممت هذه الدراسة للتحقق من خصائص مضادات الأكسدة والالتهابات للمركبات الكيميائية النباتية في مستخلص نبات طبوق العشاء ضد قرحة المعدة المُستحثة بالأسبرين في نموذج الفئران. النتائج: أشارت الفحوصات الكيميائية النباتية (HPLC, FTIR, LC-MS/MS) وجود مركبات نشطة متعددة في مستخلص نبات طبوق العشاء. وكشف الفحص النسيجي المرضي عن تلف ملحوظ في الغشاء المخاطي في المجموعة المصابة بالقرحة مقارنةً بالمجموعات المعالجة بالأوميبرازول ومستخلص النبات التي حافظت على سلامة الغشاء المخاطي للمعدة. وقد تحسن ارتشاح الخلايا الالتهابية وبنية الغشاء المخاطي للمعدة بشكل ملحوظ بعد تناول المستخلص، ويمكن أن يُعزى هذا التأثير الوقائي إلى خصائصه المضادة للأكسدة والالتهابات. الاستنتاجات: تُظهر النتائج أن مستخلص نبات طبوق العشاء يتمتع بحماية جيدة للمعدة، وقد يكون مرشحًا واعدًا للاستخدام العلاجي.

Introduction

Gastrointestinal bleeding and increased mortality and morbidity rates have been linked in recent decades with Peptic Ulcer Disease (PUD), a widely recognized condition resulting from an imbalance between chemico-physical elements damaging the gastric mucosal defense mechanism [1]. There are many pathogenic factors of gastric ulcers, such as alcohol drinking (AD), smoking, nonsteroidal anti-inflammatory drugs (NSAIDs), high-fat diet, stress and *Helicobacter pylori* infection [2]. As a common type of upper gastrointestinal tract chronic disease all over the world, gastric ulcers frequently accompanies severe complications such as massive hemorrhage, perforation, obstruction and cancer [3,4]. Gastrointestinal lesions in the upper alimentary tract are well-known side-effects of aspirin and other non-selective NSAIDs. There are two mechanisms in which aspirin can injure the stomach. The first involves direct irritation of the stomach mucosa, and the second involves inhibition of the cyclooxygenase enzyme [5]. Nowadays, therapies for gastric ulcers include antacid medications that neutralise gastric acid, cytoprotective medications that prevent gastric mucosal cell apoptosis, and acid-secretion inhibitors that block gastric acid secretion. However, the majority of these medications have adverse effects such as joint discomfort, irregular heartbeat, hemopoietic alterations, gynecomastia, impotence, and systemic alkalosis [6]. Numerous studies to investigating various medications with minimal side effects are emerging these days [7]. Indian medicinal herbs and their byproducts have long been a valuable resource for treating stomach ulcers [8]. *Pterospermum acerifolium* (L) Willd is commonly known as “Bayur Tree”, “Dinner plate tree”, (“Kanakchampa” and “Muchukunda”). It is distinguished for its striking foliage, captivating flowers, and cultural significance in South Asia [9]. This unique and endemic *Pteronrospermum* has attracted research attention, conservation efforts, and popular interest since long ago. Apart from its phytostructure, this species is also significant because of medicinal values and uses for the cure of different diseases [10]. Previous studies reported that this plant has strong anti-inflammatory and antioxidant activities, which may act as a major antioxidant or radical scavenger or free radical scavengers or inhibitors [11]. The highest radical scavenging activity was displayed by ethyl acetate fraction of *Pterospermum acerifolium* leaves (EAF) in all models in vitro. EAF also demonstrated a strong anti-inflammatory effect in both *in vivo* and in-12 models, supporting the plant's traditional use [12]. Moreover, a 95% ethanolic

extract of *Pterospermum acerifolium* flowers has been reported to possess exceptional ulcer-preventive qualities [13].

Materials and methods

This experimental study was conducted in the laboratories of the College of Pharmacy, University of Baghdad, using both in vitro and in vivo models, from October 2024 to May 2025.

Chemicals and reagents

Certified reference standard acetylsalicylic acid (aspirin) used for gastric ulcer induction, along with absolute methanol, carbon tetrachloride (CCl₄), ether, 10% formaldehyde, liquid paraffin, paraffin wax, hematoxylin, eosin, and xylene, were all obtained from Sigma-Aldrich (St. Louis, MO, USA). Additional constituents, including normal saline and vegetable oil, were purchased from the local market.

Plant extract preparation

The plant material (*Pterospermum acerifolium*) was collected in June 2024 from an orchard in Baghdad and identified by botanical specialists at the College of Science, University of Baghdad. To remove the waxes and lipids, the plant was shade-dried at room temperature (25 °C) for 3 weeks, pulverized in an electric mill, and then 250 g of the powder was homogenized with n-hexane for three days. Plant powder was dried at room temperature, and the sample was extracted with 85% methanol in a Soxhlet apparatus until the extract turned colorless. After filtering and concentrating with a rotary evaporator, the residue extract was suspended in 250 milliliters of distilled water. The residue of the methanolic extract was obtained by further partitioning the extract with 250 ml of ethyl acetate, using a separatory funnel, three times. It was then dried over anhydrous sodium sulfate, filtered, and concentrated using a rotary evaporator [14]. To identify secondary metabolites, a preliminary phytochemical screening was performed on the ethyl acetate fractions of *Pterospermum acerifolium*. Flavonoids and phenols were estimated in the extract using standard qualitative techniques.

Identification of compounds by High Performance Liquid Chromatography

HPLC is one of the most commonly employed and very sensitive analytical instruments used for qualitative and quantitative determination of pharmaceutical substances. Optimizing and validating HPLC methods imparts more importance to the pharmaceutical industry, as these techniques play a significant role to drug discovery, formulation development and manufacturing with obtaining product quality, safety and efficacy [15,16]. HPLC analysis was performed to isolate the active constituents from the ethyl acetate fraction using an isocratic elution method on a C18 column (250 × 4.6 mm, 5 µm particle size). The mobile phase was a 1:1 mixture of methanol and water containing 5% formic acid. The system worked at a flow rate of 0.75 mL/min, with detection at 254 nm and an injection volume of 20 µL. Specimen retention durations were compared with those of standard materials under the same experimental parameters. HPLC analysis was performed on the ethyl acetate extract to separate and identify the chemical constituents present, particularly gallic acid and vanillic acid, which are known for their strong antiulcer activity [17,18].

Characterization of the isolated compounds

The following list of chromatographic and spectroscopic methods has been used to characterise the separated gallic acid and vanillic acid:

1. Analysis of spikes by analytical HP

Using 10 ppm/100 μ L of the isolated samples, mixed with the same quantity of their standards for reanalysis by HPLC, and comparing the isolated compounds with their standards based on retention times under identical conditions allowed for this identification [19].

2. Fourier transform infrared spectroscopy (FTIR)

The functional groups of the extracted gallic acid and vanillic acid were identified by FT-IR spectra obtained with the Shimadzu (Japan) instrument, and the results were further validated to determine the functional groups of the separated constituents. The KBr disc was used, and the scanning range was between 4000 and 400 cm^{-1} .

3. Liquid chromatography mass spectrometry (LCMS/MS)

At the Jordan University of Science and Technology, the data of LC-MS/MS analysis was recorded using the Shimadzu (Japan) instrument model in accordance with the following protocols: The GL-Science has a 5 μ L volume, a 1 ml/min flow rate, a 25-minute run duration, an ESI positive ionisation mode, a 50-800 m/z scan range, and a 5500V ion source voltage. Water and 0.1% formic acid, together with a 50:50 (v/v) acetonitrile: methanol mixture, comprised the isocratic mobile phase [20].

Experimental design of murine models.

The animal facility was the source of the CD1 (ICR) albino mice (containing 25 g). Mice were randomly assigned to one of four groups (n = 10 per group) to receive normal control (Phosphate Buffered Saline), ulcer control (aspirin only 500 mg/kg), standard drug (aspirin + omeprazole 10 mg/kg), and one plant extract fraction (20 mg/kg)[21]. To exacerbate gastric injury, mice were fasted for 24 hours to empty their stomachs, which stimulates gastric acid secretion [22]. Mice were continuously given acetylsalicylic acid (500 mg/kg) on days 14, 21, and 28 after it was delivered orally for 10 days to cause gastric ulcers. On day 11, mice received omeprazole (10 mg/kg) and the plant extract fraction (20mg/kg) orally once daily until day 31. Following the experimental procedure, the mice were euthanized, and their stomach tissues were processed for histological analysis and ulcer assessment on day 32 [21]. Ethical approval was obtained from the Research Ethics Committee with approval number: RE022418R on 13/10/2024

Histopathological analysis

Tissues were fixed in 10% formaldehyde, dehydrated through a series of graded ethanol concentrations, and cleared with xylene. Samples were embedded in paraffin, sectioned at 5 μ m, and mounted on slides. Deparaffinization was done using heat and xylene, followed by rehydration and staining with hematoxylin and eosin. Finally, slides were dehydrated, cleared, and covered for microscopic analysis [23].

Results and discussion

The yield of ethyl acetate extract obtained by the Soxhlet extraction was 2.6 g. Since ethyl acetate, with moderate polarity, facilitates the extraction of semi-polar bioactive components, it was selected for further examination. For compound profiling, the analysis of ethyl acetate fraction by HPLC indicated the presence of some phenolic and flavonoid compounds in *Pterospermum acerifolium* which are detected at the following retention time: protocatechuic acid (9.00), vanillic acid (18.11), gallic acid (11.90), quercetin (3.00), luteolin (15.50), rutin (6.10), as shown in Figure 1.

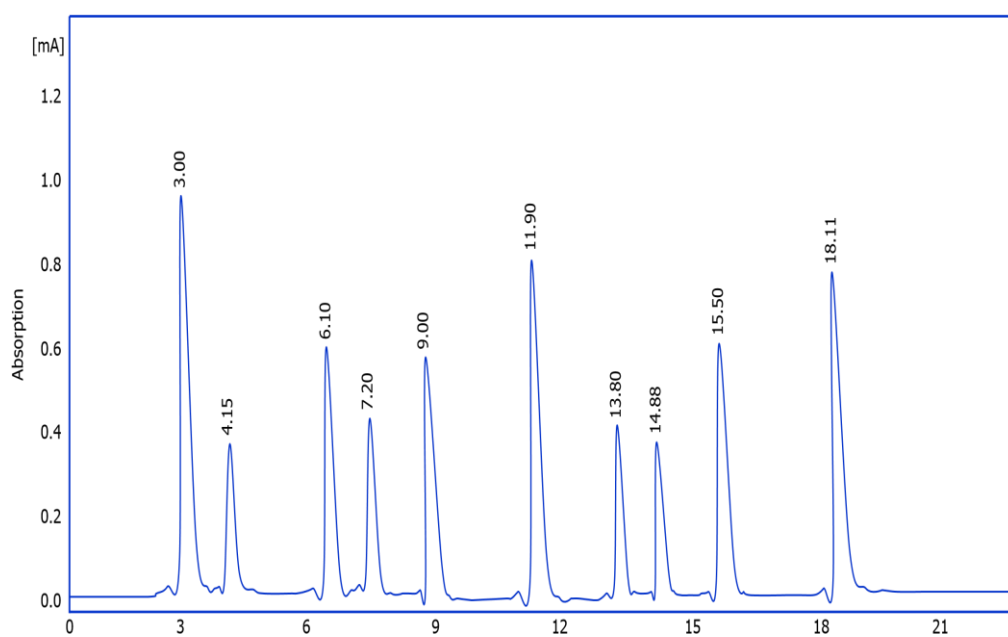


Figure 1:. HPLC chromatogram for ethyl acetate fraction

Based on the retention time (min^{-1}) of isolated compounds by preparative HPLC, the retention time of isolated gallic acid matches the retention time of gallic acid standard under the same conditions as shown in Figures 2 and. The retention time of vanillic acid standard matches the retention time of the standard of vanillic acid, as shown in Figures 4 and 5.

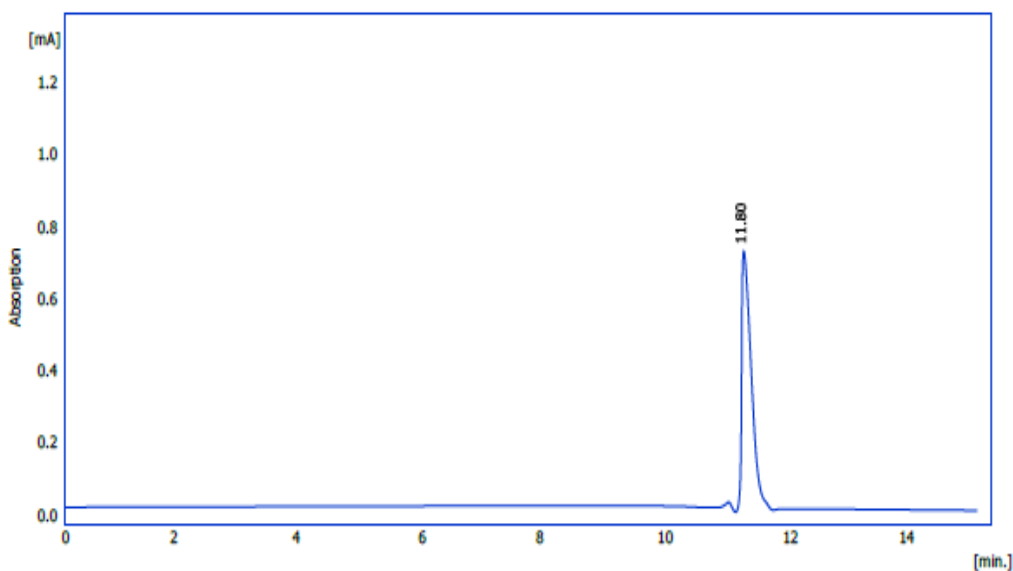


Figure 2: HPLC chromatogram for isolated gallic acid

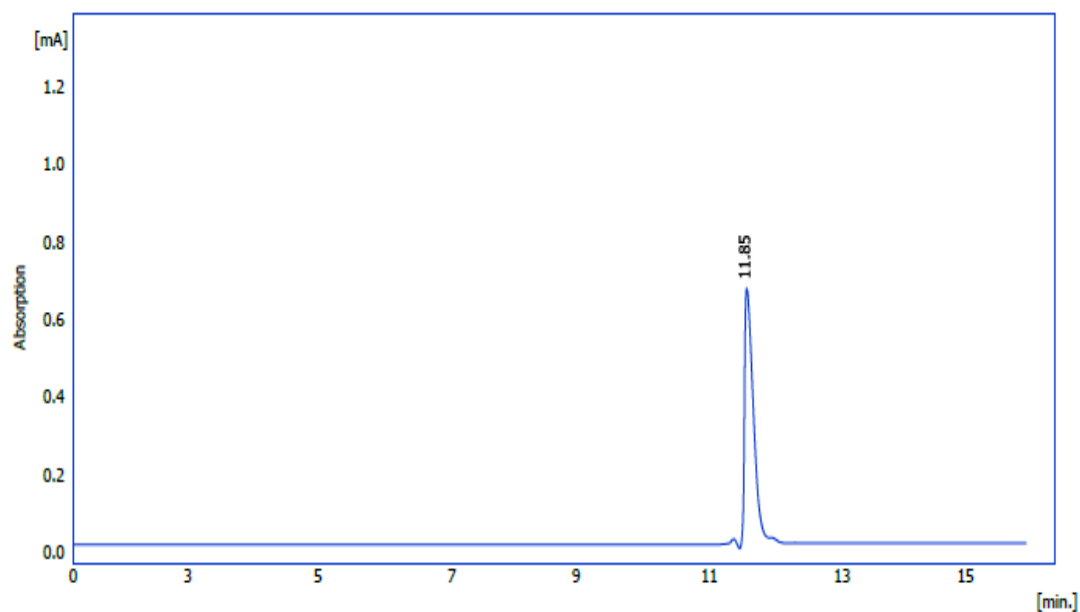


Figure 3: HPLC chromatogram for standard gallic acid

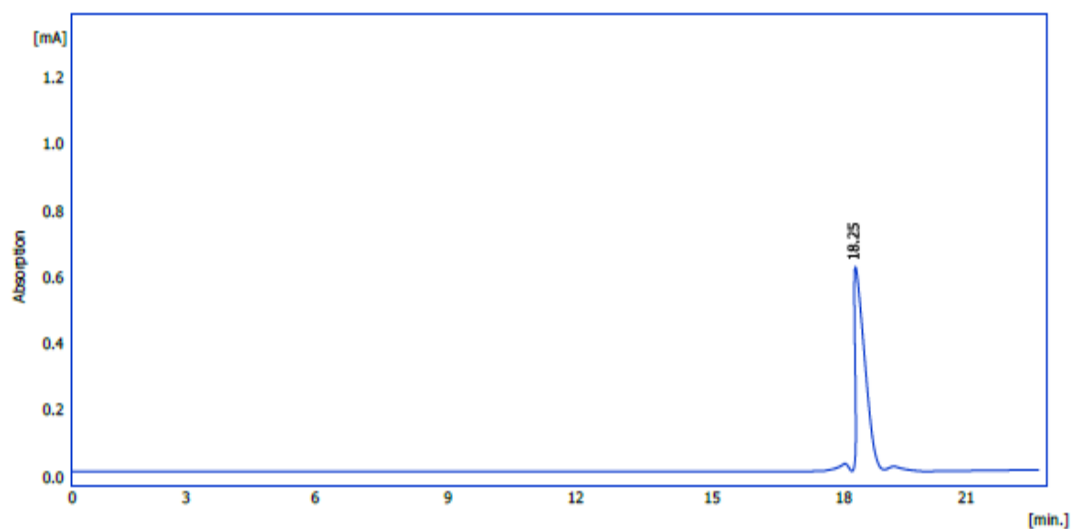


Figure 4: HPLC chromatogram for isolated vanillic acid

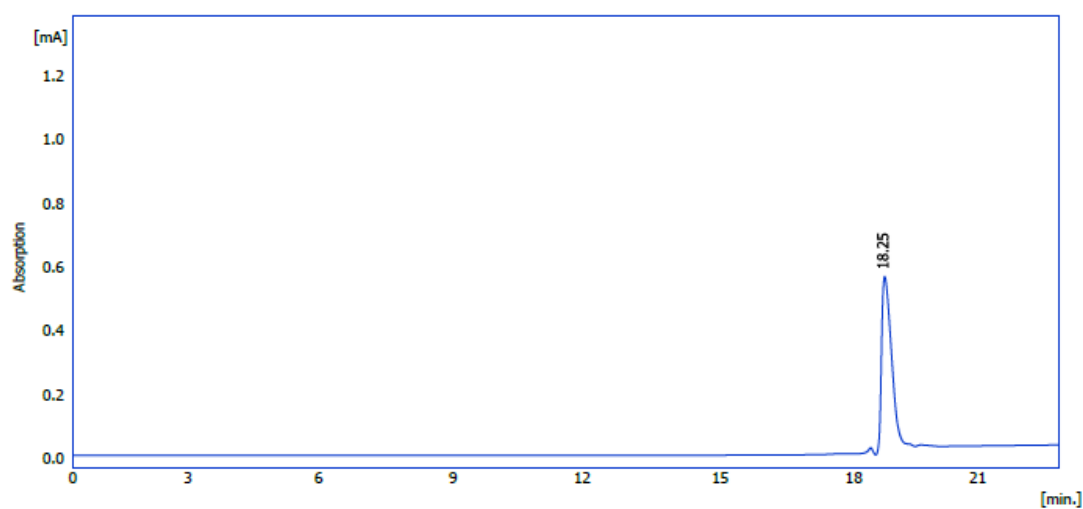


Figure 5: HPLC chromatogram for standard vanillic acid

Characterization of the isolated compounds

Spiking analysis by analytical HPLC

The spiking method involves adding a known amount of the standard to the isolated compound, then re-examining the mixture by HPLC, and identifying the isolated compound by comparing its retention time to that of the known standard [19]. When mixing the isolated compounds (gallic acid and vanillic acid) with their standards, they show an increase in area, which confirms the presence of these compounds in the ethyl acetate fraction of *Pterospermum acerifolium*, as shown in Figures 6 and 7.

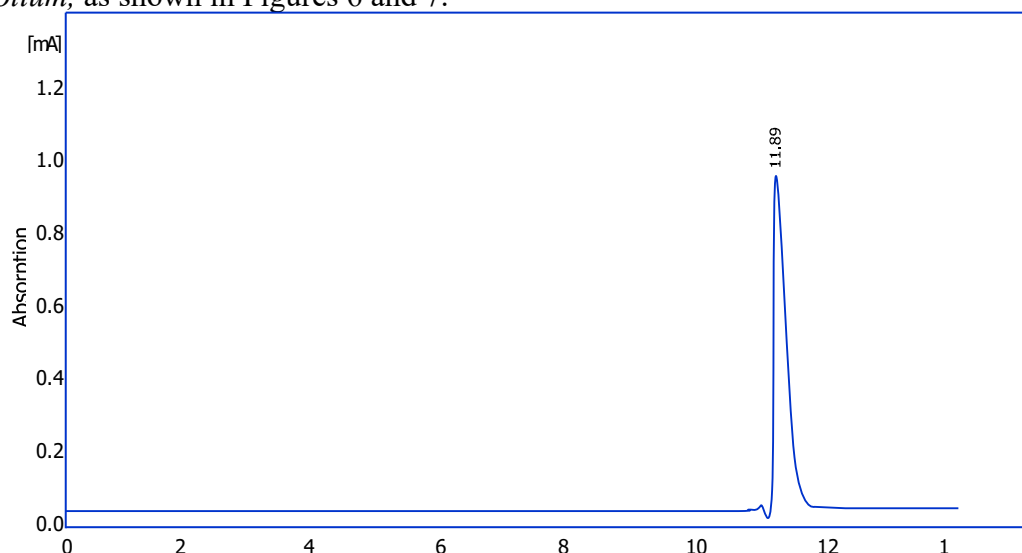


Figure 6: Spiking analysis of gallic acid

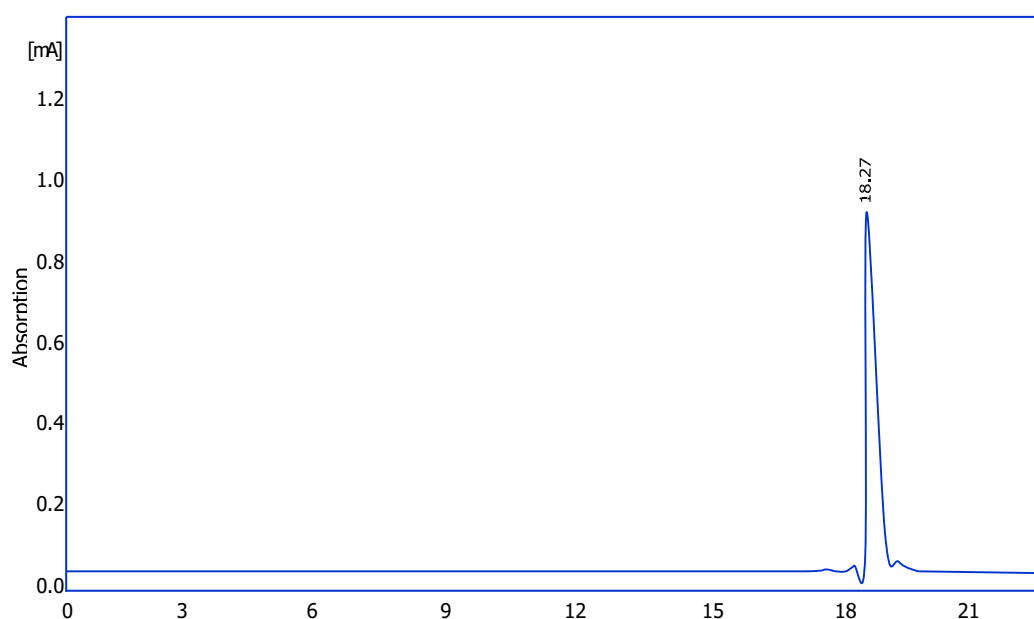


Figure 7: Spiking analysis of vanillic acid

Fourier transform infrared spectroscopy (FTIR)

Gallic acid was confirmed to be polyhydroxylated by the large absorption band at 3283 cm^{-1} in its FTIR spectra, which was ascribed to O–H stretching vibrations of hydroxyl groups. While intramolecular hydrogen bonding may be linked to the weak peak at 2662 cm^{-1} , the band at 3065 cm^{-1} was connected with aromatic C–H stretching. Strong C=O stretching of the carboxylic acid group was indicated by an absorption at 1704 cm^{-1} , and the band further supported the presence of the benzoic acid framework at 1613 cm^{-1} , which was caused by aromatic C=C stretching vibrations as shown in Figure 8. Similarly, O-H stretching was

identified as the large peak at 3482 cm^{-1} in the vanillic acid FTIR spectrum, while aromatic C-H stretching was identified as the band at 3039 cm^{-1} . Its structural characteristics were consistent with the signal at 2851 cm^{-1} , which was attributed to aliphatic C-H stretching from the methoxy group. The C=O stretching vibration of the carboxyl group was confirmed by a high absorption at 1685 cm^{-1} , while aromatic C=C stretching was indicated by the band at 1592 cm^{-1} , as shown in Figure 9, and the interpretation of bands is shown in Table 1. All the above data confirm the structure of gallic acid and vanillic acid that's found in the ethyl acetate fraction of *Pterospermum acerifolium*

Table 1: FTIR interpretation for isolated gallic acid and protocatechuic acid

Gallic acid	
IR bands	Interpretation
3283	OH stretching of phenol
3065	C-H stretching of aromatic
2662	OH of the carboxylic acid group
1704	C=O stretching for the carboxyl group
1613	C=C aromatic stretching
1435	OH bending for the phenolic
1322	C-O stretching for the phenol group
Vanillic acid	
3482	OH stretching for the phenol group
3039	C-H stretching for aromatic ring
2851	C-H stretching for the methoxy group
1685	C=O for carboxyl group
1592	C=C for aromatic stretching

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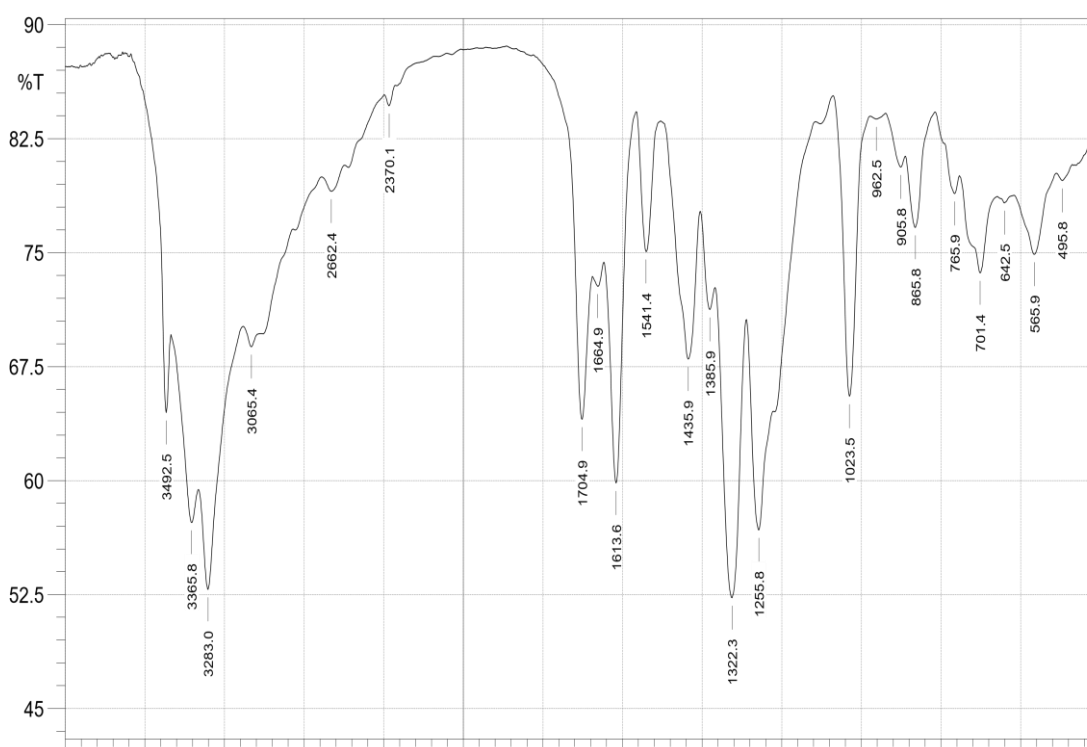


Figure 8: FTIR spectroscopy for isolated gallic acid

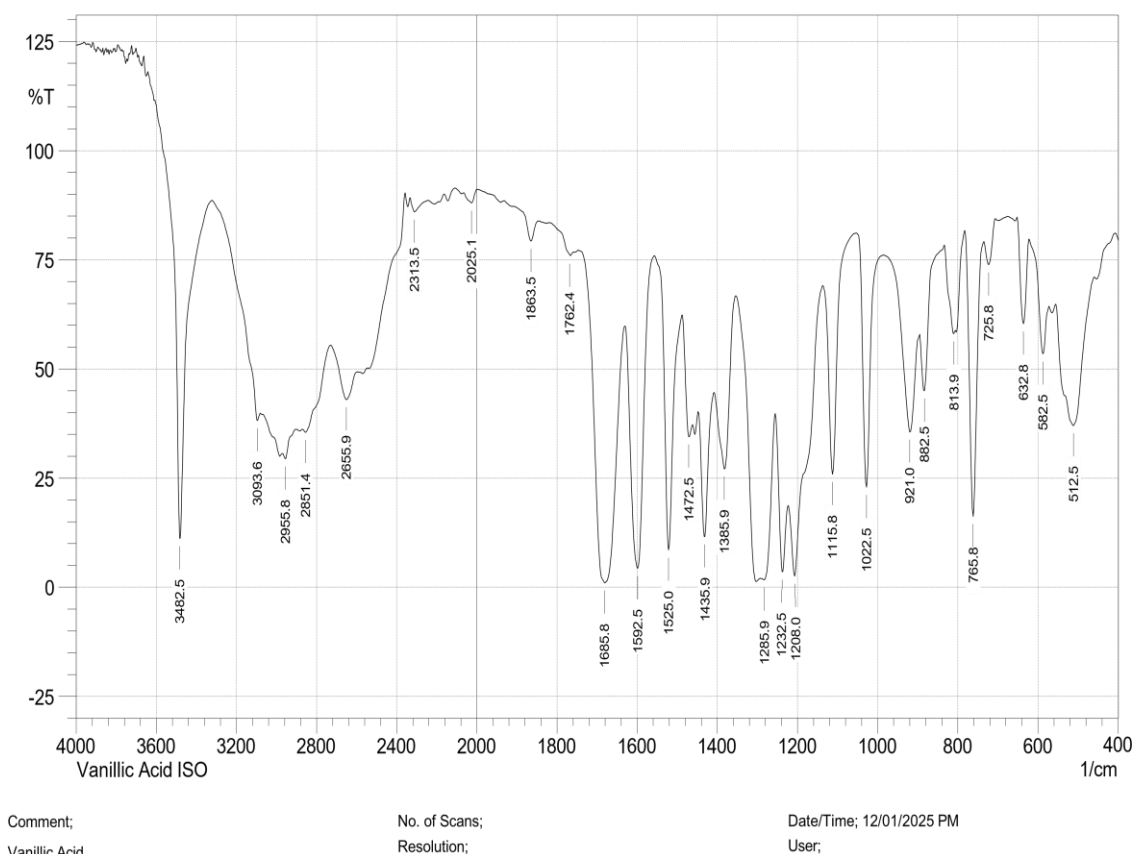
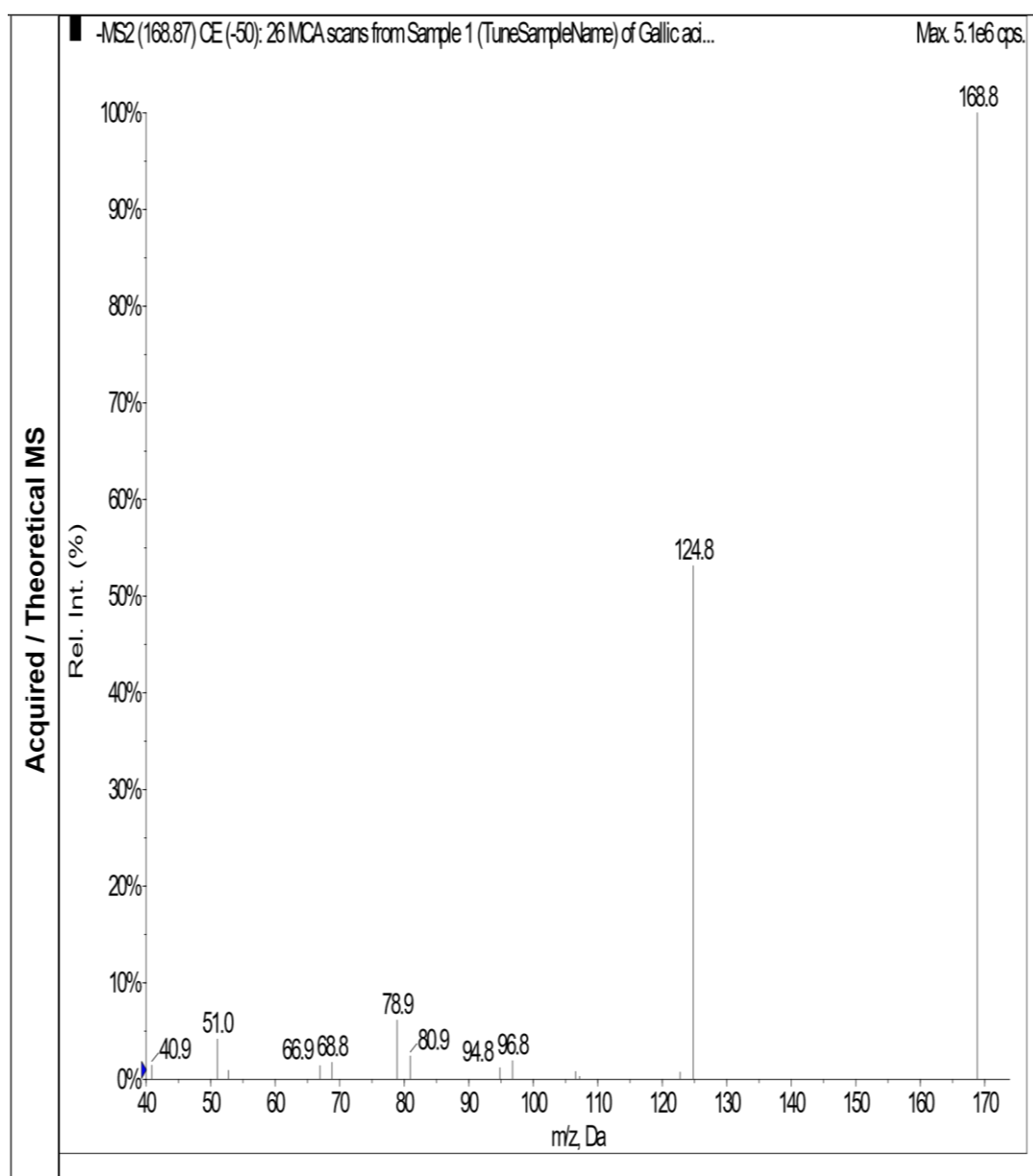


Figure 9: FTIR spectroscopy for isolated vanillic acid.

Liquid chromatography mass spectrometry (LCMS/MS)

The LC-MS/MS study was approved to detect and characterize isolated (gallic acid and vanillic acid), as shown in Figures 10 and 11, respectively. LCMS/MS fragmentation of gallic acid show most prominent fragment ion was observed at m/z : 169 which is parent ion of deprotonated gallic acid ($C_7H_5O_5^-$), m/z : 124.8 referred to loss of CO_2 ($C_6H_4O_3^-$), m/z : 94.8 referred to fragment of the aromatic ring after further breakdown ($C_6H_2O_2^-$), m/z : 78.9 referring to benzene-like ring fragment ($C_6H_6^-$). While LCMS/MS fragmentation of vanillic acid shows the most prominent fragment ion at m/z : 166.8, referred to the deprotonated vanillic acid ($C_8H_7O_4^-$), m/z : 151.6 referred to the loss of CH_3 from the methoxy group ($C_7H_5O_4^-$), m/z : 122.9, referred to the loss of $COOH$ and CH_3 ($C_6H_6O_2^-$). These data confirmed the presence of several phenolic acids, including gallic acid and vanillic acid, identified by their characteristic fragmentation patterns. These compounds are well recognized for their antioxidant and anti-inflammatory properties [17,18], which may underlie the gastroprotective effect observed in this study. Their detection is consistent with previous phytochemical reports and further validates the traditional use of the plant in gastric disorders.

**Figure 10:** LC-MS/MS of isolated gallic acid

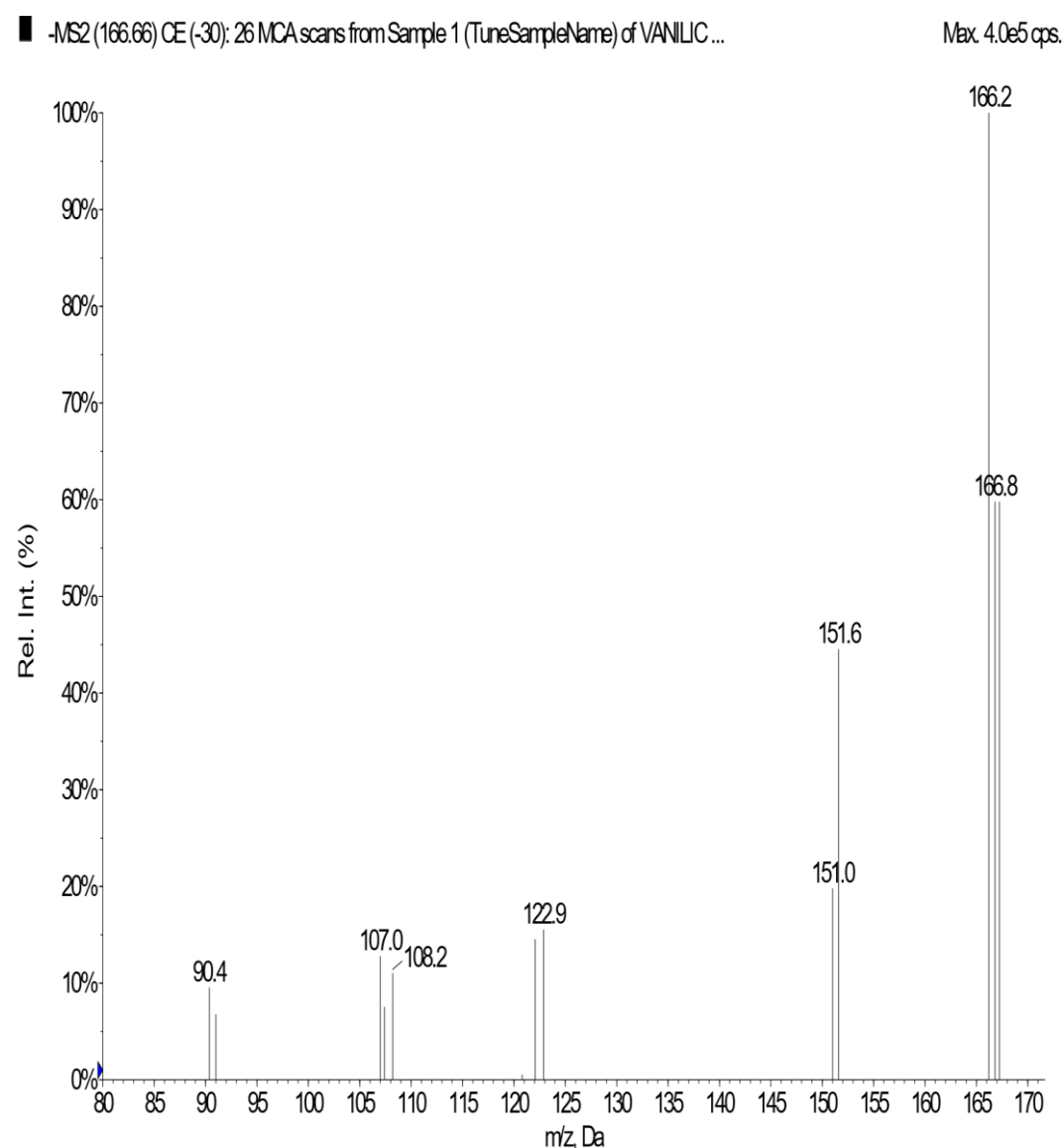


Figure 11: LC-MS/MS fragmentation of isolated vanillic acid.

Histopathological analysis

The histopathological figure of the stomach in the control group shows intact gastric mucosa and a submucosal layer, indicating healthy tissue architecture without signs of inflammation or ulceration (Figure 12). In contrast, the gastric tissues of the aspirin-induced mice group showed marked degeneration, necrosis, and atrophy of the lining cells of gastric glands, with marked inflammatory cell infiltration (Figure 13).

In the omeprazole treatment group (Figure 14), gastric tissue showed improvement. There is partial cell degeneration with focal necrotic lesions, accompanied by reduced inflammatory cell infiltration, compared with the aspirin-induced ulcer group (Figure 15).

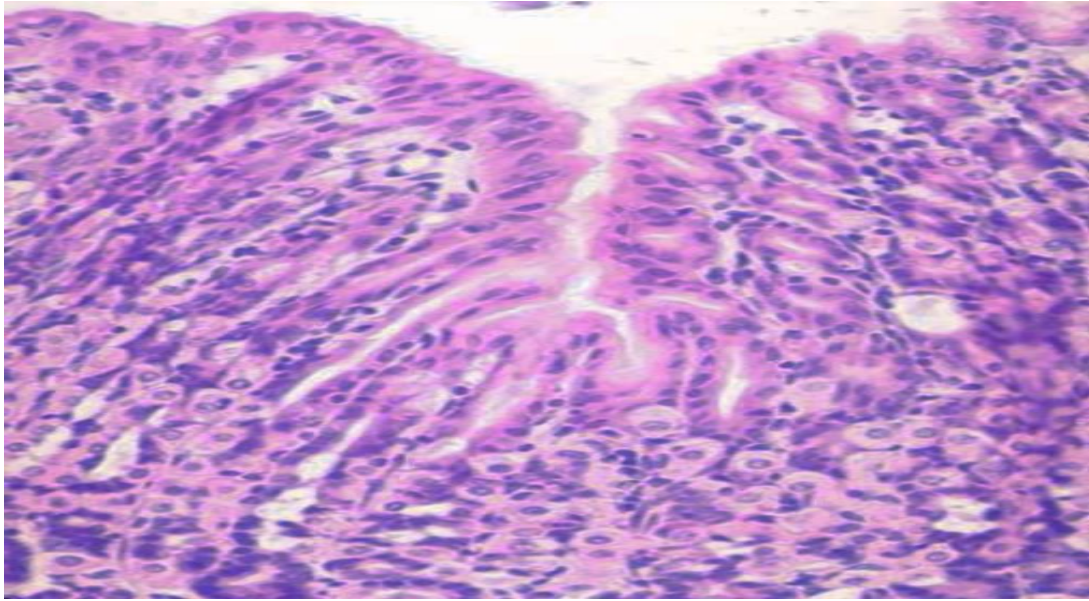


Figure 12: Cross-section of gastric tissue in the healthy control mice group stained with H&E, showing intact gastric mucosa and submucosal layer (under light microscope 40 x)

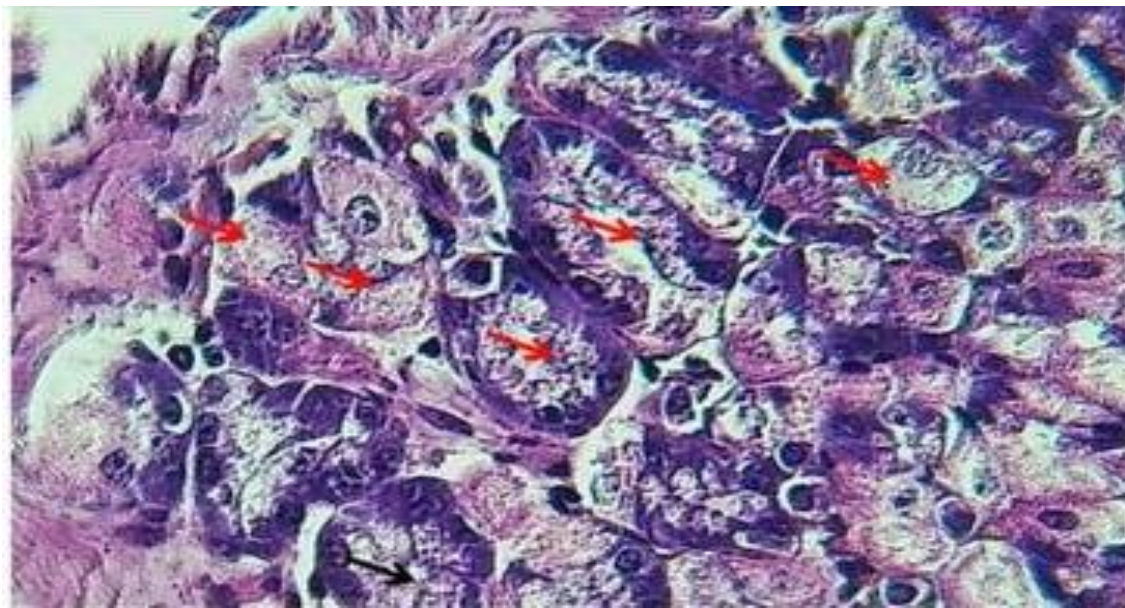


Figure 13: Cross-section of gastric tissue in the aspirin-treated mice group (induction group) stained with H&E, showing marked degeneration and necrosis and atrophy of lining cells of gastric glands (red arrow) and chief cells (black arrow) (under light microscope 40 x).

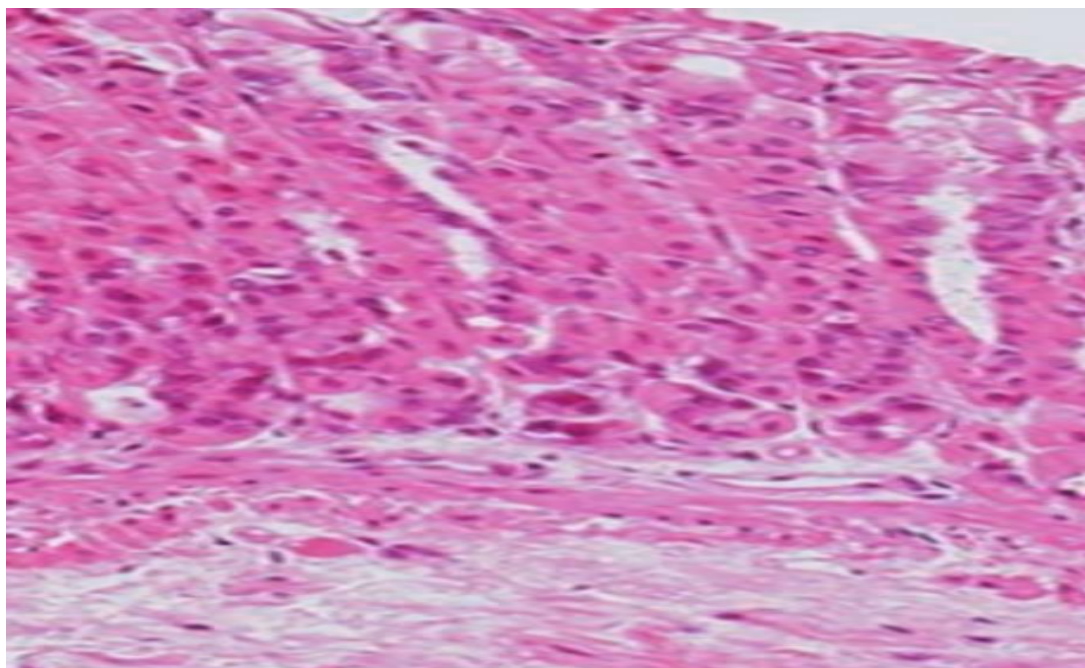


Figure 14: Cross-section of gastric tissue in the omeprazole-treated mice group stained with H&E, showing improved gastric mucosa and submucosal layer (under light microscope 40 x).

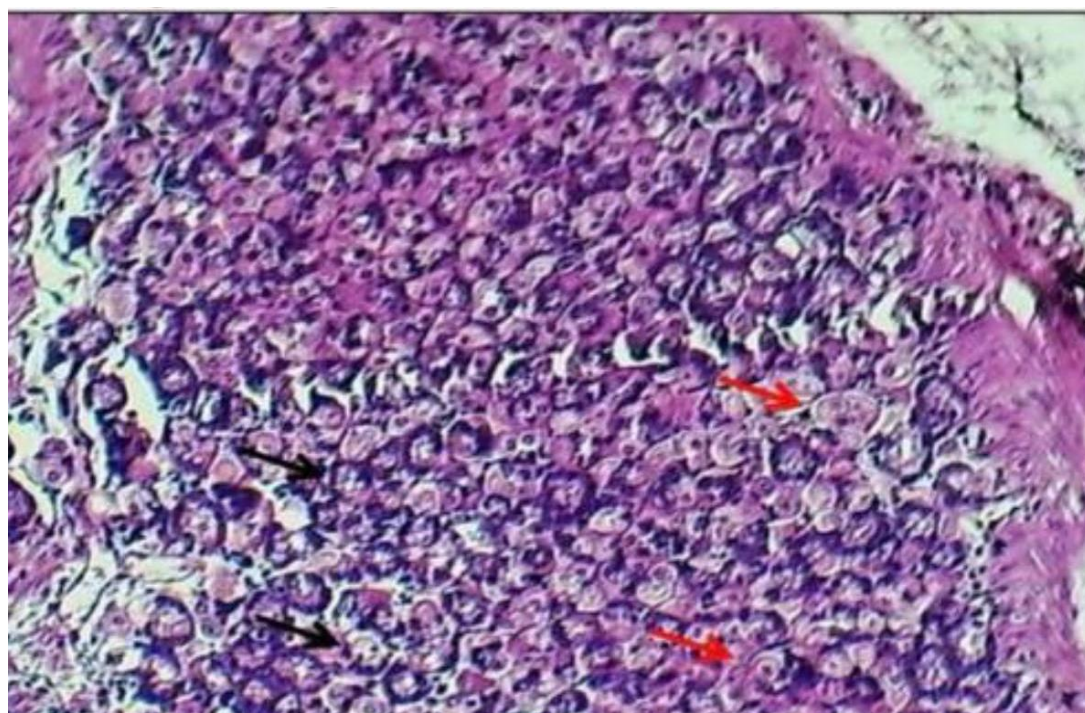


Figure 15 : Cross-section of gastric tissue in the ethyl extract-treated mice group (induction group) stained with H&E, showing marked improvement and less inflammatory cell infiltration (red arrow) and chief cells (black arrow) (under light microscope 10 x).

The histopathological examination provided critical insights into the protective effects of the *Pterospermum acerifolium* ethyl acetate extract on gastric mucosal integrity in aspirin-induced ulceration. Gastric tissue sections from the control group showed typical histoarchitecture as well as intact mucosal and submucosal layers, absence of necrosis, no inflammatory infiltration (Figure 12) so that gastric morphology was normal and unaltered. In

contrast, the ulcer-induced (aspirin-treated) group showed remarkable degeneration and necrosis of gastric glandular epithelium, mucosal atrophy and serious inflammatory cells infiltration (Figure 13). These data further affirm aspirin's well- documented ulcerogenic actions, largely because of direct mucosal irritation and inhibition of COX enzymes subsequently resulting in decreased prostaglandin synthesis and impaired defense of the mucosa [24]. For the omeprazole-treated mice group, degeneration of gastric epithelial cells was partially accompanied by small areas of necrosis and reduced infiltration of inflammatory cells compared with the aspirin-induced mouse group. Our results could be supported by another study that has shown omeprazole treatment resulted in significant gastroprotection in the aspirin-induced gastric ulcer model. Apart from its anti-inflammatory and antioxidant features, omeprazole decreases the ulcer's occurrence, increases gastric mucus secretion and assists in preventing mucosal defensiveness [25]. Notably, there were observable improvements from the gastric tissues of the *Pterospermum acerifolium* extract-treated group. There was still a light partial loss of cells and focal necrosis area, but contrary to the ulcer-received group, they were much milder.

It is suggested that the phenolic and flavonoid compounds separated from the extract fraction would have antioxidant, anti-inflammatory action against aspirin-induced ulceration in mouse. The present findings are consistent with earlier reports indicating the potent of anti-inflammatory and antioxidant activities of *Pterospermum acerifolium* extract. Methanolic, ethanolic and ethyl acetate extracts obtained from various parts of plants (i.e., bark leaves, flowers) have showed a remarkable anti-inflammatory action in vivo/vitro model systems, specifically inhibiting the principal inflammatory mediators like histamine and serotonin [26]. Additionally, the plant exhibited strong antioxidant properties, with the ethyl acetate fraction showing the highest free radical scavenging activity in multiple in vitro assays, including DPPH, nitric oxide, and superoxide anion scavenging [27].

Conclusion

The present investigation has brought out the gastroprotective activity of *Pterospermum acerifolium* extract by maintaining the normal histological profile of gastric mucosa and preventing ulcer formation. The protective effect is probably due to its antioxidant and anti-inflammatory ingredients. These observations underline its possible role as a natural biologically active candidate against gastric ulcers and need to be explored at molecular and mechanistic level.

Recommendation

Additional investigations are necessary to explore the molecular mechanisms of *Pterospermum acerifolium* in antiulcer activity, such as modulation of inflammatory pathways, oxidative stress and gastric mucosal defense. Dose optimization and in vivo safety profiling with different doses of ethyl acetate fraction are needful to establish the ideal therapeutic dose as well as anticipate any toxicological effects associated with prolonged use.

Acknowledgements

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Conflict of interest

The authors have no conflicts of interest to declare.

Ethical issues

The study was conducted in accordance with institutional and national ethical guidelines and was approved by the Research Ethics Committee (Approval No. RE022418R, 13/10/2024).

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