

Phytochemical analysis and gastroprotective effect of *Stellaria media* (L.) Vill. methanolic extract on piroxicam-induced gastric ulcer in Wistar rats

Abdul Aleem

University of Agriculture Faisalabad

Bilal Aslam (✉ cba933@gmail.com)

University of Agriculture Faisalabad <https://orcid.org/0000-0003-3383-8961>

Muhammad Bilal Alim

University of Sargodha Faculty of Medical and Health Sciences

Asif Hussain

University of Agriculture Faisalabad <https://orcid.org/0000-0002-8026-6110>

Muhammad Naeem Faisal

University of Agriculture Faisalabad

Wafa Majeed

University of Agriculture Faisalabad

Research Article

Keywords: Gastric ulcer, Piroxicam, *Stellaria media*, HPLC, FT-IR

Posted Date: February 23rd, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-1835268/v2>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Stellaria media (L.) Vill. is widely distributed throughout the world and is traditionally used to treat inflammatory, respiratory, cardiovascular, and gastrointestinal diseases. This study was designed to phytochemically characterize and investigate the anti-ulcer activity of methanol extract of *S. media* (SME) in piroxicam (PRX)-induced gastric ulcer in rats. The plant extract was subjected to qualitative as well as quantitative analysis (HPLC and FT-IR) to elucidate the phytochemical composition. *In vitro*, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was done to determine the antioxidant capacity. In 14 days of animal study, PRX (30 mg/kg, i.g.) was co-administered with omeprazole (OMP; 20 mg/kg, i.g.) as a standard gastroprotective drug and SME at 150, 300, and 450 mg/kg, i.g., respectively. The gastric pH, acid volume, acidity, ulcer score, hematological parameters, and serum levels of oxidants/antioxidants were determined along with histopathological studies of gastric tissue. Phytochemical analysis showed the presence of considerable phenolic and flavonoid contents which was corroborated with a significant DPPH radical scavenging (IC₅₀: 27.94 µg/mL) activity of extract. Administration of SME at 150, 300, and 450 mg/kg exhibited a dose-dependent gastroprotective effect evidenced by an increase in gastric pH and acidity but a decrease in gastric acid volume, gastric ulcer score, and ulcer index. Treatment with SME normalized the altered hematological parameters and reduced the oxidative stress by decreasing serum levels of TOS and MDA, and increasing the TAC and CAT levels. In addition, histopathological findings supported the aforementioned results. This study concludes that *Stellaria media* has promising gastroprotective activity against PRX-induced gastric ulcer.

Highlights

- *S. media* contains considerable polyphenol and flavonoid contents.
- *S. media* demonstrated significant free radical scavenging activity.
- *S. media* extract protects against piroxicam-induced gastric mucosal injury.
- *S. media* extract ameliorates piroxicam-induced oxidative stress

Introduction

Gastric ulcer is an inflammatory and necrotizing condition that affects nearly 5-10% of the global population (Lanas and Chan 2017). Its main characteristics include erosions of the gastric wall, perforations, and bleeding due to an imbalance between defensive factors such as mucin, bicarbonate, prostaglandin, nitric oxide, growth factors, and mucosal blood flow and damaging factors like infection (*Helicobacter pylori*), gastric acid, and pepsin (Ray et al. 2020; Kamada et al. 2021). Besides that, smoking, chronic alcohol use, stress, long-term use of non-steroidal anti-inflammatory drugs (NSAIDs), and bad dietary habits also contribute to the pathogenesis of gastric ulcers. It is a global health problem, as each year 4.6 million individuals are diagnosed with gastric ulcers (Kuna et al. 2019).

Piroxicam (PRX) is an NSAID that is effectively used to treat fever, pain, and inflammatory conditions like osteoarthritis, rheumatoid arthritis, and post-traumatic and post-operative inflammation (Saganuwan 2017). Despite its therapeutic uses, PRX is known to induce gastric ulcers. The pathogenesis of PRX-induced gastric ulcer includes its non-selective inhibition of cyclooxygenase enzymes 1 and 2 (COX-1, COX-2), which subsequently blocks the production of prostaglandins (PGs) and leads to decrease in secretion of mucus and bicarbonate, reduced blood flow, and epithelial damage due to alteration in microvascular structures and excessive production of reactive oxygen species (ROS) as well as reduced activity of antioxidant enzymes (Abdeen et al. 2019; Abd-Alla et al. 2022).

Traditional therapies for the treatment of gastric ulcers have limitations due to the limited efficacy and unfavorable side effects of currently available drugs for gastric diseases. Furthermore, the relapse of disease after exhaustive treatments and the adverse effects of drugs have drawn attention to the search for novel and cost-effective agents that offer better protection against gastric diseases along with fewer adverse effects. However, several studies reported the effective use of herbs in traditional medicine, and they have exhibited promising outcomes in these pathological conditions (Gupta et al. 2021; Kavitt et al. 2019).

Stellaria media L. (Vill.), popularly known as 'Chick week', belongs to the Caryophyllaceae family. It is a perennial small shrub and is widely distributed in cold and temperate regions of Africa, North America, Europe, and Asia. Different parts of *S. media* are commonly used in ethnomedicine to cure measles, asthma, diarrhea, jaundice, and gastrointestinal disorders. It also decreases swelling and is used as a plaster for fractured bones (Oladeji and Oyebamiji 2020). The antioxidant, anti-microbial (Teuşdea et al. 2021), anti-diabetic, anti-obesity, cardioprotective (Khan et al. 2019; Demján et al. 2022), and neuroprotective (Ahmad et al. 2022) activities of *S. media* have been reported. However, the efficacy of *S. media* on drug-induced gastric toxicity has not yet been investigated. Therefore, the present study was planned to evaluate the gastroprotective effect of methanol extract of *Stellaria media* on PRX-induced gastric ulcer in rats.

Materials And Methods

Drugs, chemicals, and reagents

Drugs including piroxicam (PRX) from Pfizer®, Pakistan, and omeprazole (OMP) from Getz Pharma®, Pakistan, were purchased. Chemicals and reagents of analytic grade such as 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, gallic acid, catechin, aluminum chloride (AlCl₃), Folin-Ciocalteu reagent, and Topfer's reagent were procured from Sigma-Aldrich®, USA.

Plant collection

S. media (whole plant) used in this research was collected from the Punjab Forest Research Institute (PFRI), Faisalabad, Pakistan. The plant was authenticated by a taxonomist, and the specimen was

submitted to the Department of Botany, University of Agriculture, Faisalabad (UAF), Pakistan, for future reference (herbarium number: 283-1-22).

Preparation of methanolic extract of *Stellaria media*

The triple maceration method was adopted for the preparation of methanolic extract of *S. media* (Zhang et al. 2018). The whole plant was thoroughly rinsed, shade dried, and then pulverized with the help of a mechanical grinder. The powdered specimen (250 g) was macerated in 2.5 L of methanol for 72 h with vigorous shaking at regular intervals. The mixture was filtered through muslin cloth and filter paper (Whatman filter paper 1). Methanol was evaporated at 55°C using a rotary evaporator (Heizbad Hei-VAP®, Germany), and the obtained concentrated extract was weighed to calculate the extraction yield (Eq. 1).

$$\text{Extraction yield (\%)} = \frac{\text{Weight of extract (g)}}{\text{Weight of powdered plant (g)}} \times 100 \quad (1)$$

Preliminary phytochemical screening

Qualitative phytochemical tests were performed to confirm the presence of various primary and secondary phytoconstituents including phenols, flavonoids, glycosides, alkaloids, steroids, saponins, tannins, carbohydrates, proteins, and fixed oils according to previously described methods (Ajuru et al. 2017).

Estimation of total phenolic content

The Folin-Ciocalteu method was adopted to determine the total phenolic content (Sultana et al. 2009). In short, 50 mg of extract mixed with 7.5 mL of distilled water was added to 500 µL of Folin-Ciocalteu reagent and kept in dark for 10 min. Further, the mixture was mixed with 1.5 mL of 20% Na₂CO₃ solution, heated at 40°C for 20 min and immediately cooled. The absorbance of reaction mixture was checked at 765 nm using a spectrophotometer (Shimadzu®, Japan). The phenolic content was quantified using the calibration curve of gallic acid ($y = 0.011x + 0.008$, $R^2 = 0.998$) and expressed in milligram gallic acid equivalent per gram weight of the dry extract (mg GAE/g of dry extract).

Determination of total flavonoid content

The aluminum chloride (AlCl₃) spectrophotometric method was used to quantify the total flavonoid content (Sultana et al. 2009). Briefly, 1 mL of extract was mixed with 300 µL of 5% NaNO₂, diluted with 4 mL of distilled water, and incubated in the dark for 5 min. Then, 300 µL of 10% AlCl₃ and 2 mL of 1 M NaOH were added to the mixture and further diluted with 2.5 mL of distilled water. Absorbance was measured spectrophotometrically at 510 nm and the calibration curve of catechin ($y = 0.040x + 0.009$; R^2

= 0.999) was used to calculate the flavonoid content and expressed in milligram catechin equivalent per gram weight of dry extract (mg CE/g of dry extract).

High-performance liquid chromatography (HPLC) analysis

HPLC analysis was performed to elucidate the polyphenol composition of methanolic extract. HPLC instrument comprised of a C₁₈ column and UV-Vis detector (Shimadzu, Japan) accompanied by the EZchrom Elite[®] software package for data acquisition was used. For sample preparation, dried extract (50 mg) was mixed with 40 mL of 60% methanol, acidified using HCl, and boiled for 2 h at 90°C. The mobile phase consisted of acetic acid (6%, v/v; pH 2.27) and acetonitrile (100%, v/v) with gradient elution: 15 min for 15, 45, and 100% of each combination. A sample (20 µL) was injected *via* the injection port. HPLC conditions including column temperature at 27°C, 1 mL/min of flow rate, and absorbance at 280 nm were maintained during analysis. Retention times and concentrations were used for the quantification of phytoconstituents (Seal 2016).

Fourier transformed infrared (FT-IR) analysis

FT-IR spectroscopy was used to determine the presence of different bioactive phytoconstituents in SME by detecting various functional groups (Hussain et al. 2021). Briefly, 10 mg of dried extract was used for sample preparation and analyzed using a FT-IR spectrometer (Spectrum TwoTM, Germany). The FT-IR spectrum was obtained at a scan range of 4000 to 500 cm⁻¹ and a resolution of 4 cm⁻¹.

1,1-diphenyl-2-picrylhydrazyl (DPPH) assay

The antioxidant capacity of SME was estimated by measuring its ability to scavenge DPPH free radicals (Subhan et al. 2021). Briefly, a diluted ethanolic solution of extract (0.5 mg/mL) and ascorbic acid (standard) were used to prepare different concentrations (3.125-50 µg/mL). About 1 mL of each sample was mixed with 2 mL of freshly prepared DPPH (0.2 mg/mL) ethanolic solution and kept in the dark for 30 min. A sample containing the aforementioned reagents except the test sample/reference was used as a blank solution. Absorbance was measured in triplicates at 517 nm and percentage inhibition values were calculated from the given formula (Eq. 2).

$$\text{Scavenging (\%)} = \left[\frac{(A_o - A_s)}{A_o} \right] \times 100 \quad (2)$$

Here, A_o and A_s are the absorbance of blank solution and reaction mixture containing extract/standard, respectively.

Experimental animals

Thirty-six female Wistar rats, ranging in age from 6 to 8 weeks and weighing an average of 170 ± 20 g, were purchased and housed at the Institute of Microbiology, UAF. Animals were acclimatized for one week

before being subjected to experimental conditions that included a 12 h light/dark cycle, $25 \pm 2^{\circ}\text{C}$ temperature, 40-60% relative humidity, free access to fresh water, and a commercially available pellet chow diet.

Ethical considerations

The experimental protocols were approved by the Institutional Bioethical Committee (IBC), UAF (D. No. 2006/ORIC). The NIH guidelines (No. 85-23, 2002) were implemented throughout the experiment.

Study design

In 14 days of the experiment, rats were allocated to six groups comprising six rats in each group ($n = 6$) and daily treated *via* oral route as Normal control received 3 mL of normal saline, Positive (Ulcer) control received PRX at the dose of 30 mg/kg (Abdeen et al. 2019) and standard treatment group was co-administered with PRX and OMP (20 mg/kg) (Berezi et al. 2017). Three groups were co-administered with PRX and SME at dose rates of 150, 300, and 450 mg/kg (Oyebanji et al. 2012; Khan et al. 2019), respectively.

Blood and organ sampling

After 14 days of treatments, rats fasted overnight and blood sampling was performed under ketamine anesthesia by cardiac puncture and stored in EDTA tubes and gel clot activator tubes for hematological and biochemical analysis. Rats were sacrificed and the stomach of all animals was collected, cut along the longer curvature, and gastric contents were transferred to glass tubes. Gastric contents were centrifuged and collected supernatants were further used to assess gastric acid secretion parameters including gastric acid volume, gastric pH, and total and free acidity. Gastric tissues were macroscopically examined to evaluate the ulcer score and ulcer index. Stomach tissues were stored in a formalin buffer solution (10% v/v) for histopathological studies.

Gastric acid secretion parameters

To evaluate the gastric acidity, the gastric contents stored in tubes were mixed with 5 mL of deionized water and centrifuged for 5 min at 5,000 rpm. The supernatants were collected, and the gastric acid volume was measured using a measuring cylinder. The pH of supernatants was determined using a digital pH meter. Gastric acidity (free and total acidity) was observed by titration method using Topfer's reagent, 0.01 N NaOH solution, and phenolphthalein as an indicator and expressed in mEq/L/24 h (Adefisayo et al. 2017).

Gastric ulcer scoring indices

Each stomach was cut along the greater curvature and rinsed with normal saline to remove any gastric contents and/or blood clots. The inner mucosal lining of each stomach was examined with the help of a magnifying glass, and gastric lesions were scored according to the 0–3 scoring method described by

Peskar et al. (2002). The ulcer score was calculated by multiplying the total number of lesions by the severity factor for each rat. The ulcer index of each rat was determined using the below-mentioned formula (Eq. 3) (Sattar et al. 2019):

$$\text{Ulcer index} = (U_n + U_s + U_p) \times 10^{-1} \quad (3)$$

U_n : Mean number of ulcers per rat, U_s : Mean severity score of rat, U_p : percentage of ulcerated rats.

The percentage of ulcer inhibition in treated groups was calculated by the formula (Eq. 4) of Akhtar and Kamal (1995).

$$\text{Ulcer inhibition (\%)} = \left[\frac{(LC-LT)}{LC} \right] \times 100 \quad (4)$$

Here, LC: length of ulcer in the positive control group, LT: length of ulcer in the treated group.

Hematological parameters

Whole blood samples were analyzed to measure red blood cells (RBCs), hemoglobin (Hb) concentration, white blood cells (WBCs), and platelets (PLTs) count using an auto-analyzer machine (Boule Medical AB[®], Sweden).

Biochemical parameters

Blood samples preserved in gel clot activator tubes were allowed to clot, centrifuged for 10 min at 3,000 rpm and the collected supernatants were used to determine the serum levels of oxidant/antioxidant markers such as total antioxidant capacity (TAC) (Erel 2004), catalase (CAT) activity (Goth 1991), total oxidant status (TOS) (Erel 2005), and malondialdehyde (MDA) (Ohkawa et al. 1979) using a spectrometer (Multiskan GO[®], Thermo Scientific, USA).

Histopathological examination

The stomach tissues of all experimental rats were washed and fixed in a neutral formalin buffer solution (10% v/v) for histological study. Tissue samples were processed with graded alcohol for dehydration, xylene for cleaning, and paraffin for tissue embedding. Tissue biopsies were sliced into 5-6 μm sections and stained with hematoxylin and eosin stains (H&E). Histopathological changes were observed using the microscope (Olympus PM-10ADS, Japan), and images were taken with a software package (TOUPCAM[®], China).

Statistical analysis

Data were analyzed using SPSS software (IBM SPSS[®] version 23.0). One-way analysis of variance (ANOVA) was applied, followed by Duncan's multiple-range (DMR) test. The significance level was set at

P 0.05, and results were presented as mean $\pm$ standard error of the mean (SEM).

Results

Preliminary phytochemical analysis of SME

The percentage yield of SME was 13.62% w/w. The qualitative phytochemical testing identified alkaloids, glycosides, flavonoids, phenolics, quinones, tannins, carbohydrates, proteins, and fixed oils in SME.

Total phenolic and flavonoid contents of SME

Total phenolic content quantified in the extract was 68.12 ± 0.10 mg GAE/g while the total flavonoid content found in SME was 41.81 ± 0.07 mg CE/g.

HPLC analysis of SME

HPLC analysis detected considerable contents of chlorogenic acid, gallic acid, hydroxybutyric acid, vanillic acid, kaempferol, salicylic acid, coumarin, and rutin in SME, as shown in Fig. 1 and Table 1.

FT-IR analysis of SME

As shown in Fig. 2 and Table 2, the FT-IR analysis revealed the presence of alcohols and phenols (~ 3356 cm^{-1} , ~ 1376 cm^{-1}), flavonoids, aromatic compounds (~ 1711 cm^{-1} , ~ 1453 cm^{-1}), lipids (~ 2922 cm^{-1} , ~ 2854 cm^{-1} , ~ 1158 cm^{-1}), and halogen compounds (~ 609 cm^{-1}).

In vitro antioxidant potential of SME

Antioxidant activity of SME was determined using the DPPH scavenging assay in comparison to ascorbic acid as a reference/standard antioxidant agent (Fig. 3). The extract showed $62.27 \pm 2.18\%$ inhibition in contrast to ascorbic acid ($80.06 \pm 1.35\%$) at 50 $\mu\text{g/mL}$. The IC_{50} values for SME and ascorbic acid were 27.94 $\mu\text{g/mL}$ and 9.35 $\mu\text{g/mL}$, respectively.

Effect of SME on gastric acid secretion

As shown in Table 3, the administration of PRX alone in positive control significantly ($P < 0.05$) increased gastric acid volume when compared to normal control. SME or OMP along with PRX significantly ($P < 0.05$) lowered the acid volume as compared to positive control. While, gastric pH of SME treated group was significantly ($P < 0.05$) higher in contrast to positive control. In comparison to positive control, administration of SME and OMP significantly ($P < 0.05$) lowered the free and total acidity. Thus, SME markedly inhibited the PRX-associated increase in gastric acid secretion.

Effect of SME on gastric ulcer scoring

The SME-treated groups indicated a significantly ($P < 0.05$) decreased ulcer score and ulcer index compared to positive control, as shown in Table 4. The ulcer inhibition in SME-treated groups was found higher at 450 mg/kg dose which was nearly similar to the gastroprotective effect of OMP used in this study.

Effect of SME on hematological parameters

Results of hematological parameters in controls and SME-treated rats are mentioned in Table 5. Administration of PRX alone (positive control) significantly ($P < 0.05$) lowered RBC count and Hb concentration in comparison to normal rats. The SME treatment showed a dose-dependent increase in RBC count and Hb concentration at the end of experiment. Rats co-administered with SME and PRX significantly ($P < 0.05$) restored WBC and PLT counts when compared to positive control. Moreover, SME (300 and 450 mg/kg) exhibited better amelioration of hematological parameters in contrast to SME (150 mg/kg).

Effect of SME on oxidative stress markers

Results revealed that PRX administration markedly imbalanced the serum levels of oxidant and antioxidant markers in rats (Fig. 4). Serum levels of TOS and MDA were significantly ($P < 0.05$) elevated in the rats who received PRX alone (positive control) compared to normal control. The SME or OMP co-administration with PRX significantly ($P < 0.05$) reduced the TOS and MDA levels. Positive control treated with PRX alone resulted in a significantly ($P < 0.05$) lower TAC level and CAT activity compared to normal control. However, administration of SME or OMP along with PRX significantly raised the TAC level and CAT activity to normalcy.

Effect of SME on gastric histology

Gastric histological images of normal, positive control, and treated groups are presented in Fig. 5. Normal control showed intact gastric histological structures, including mucosal and submucosal linings. In contrast, positive control given PRX alone indicated severe degenerative changes such as erosion of epithelium, glandular damage, along with mononuclear cell infiltration in the forestomach and fundic sections of stomach. Gastric morphology of rats co-administered with SME or OMP plus PRX revealed significant ameliorative effects compared to positive control group. In addition, SME 450 mg/kg showed better gastro-protective activity against the damaging effects of PRX.

Discussion

Piroxicam (PRX) is commonly prescribed as an analgesic, antipyretic, and anti-inflammatory drug. Despite its therapeutic benefits, chronic use of PRX is associated with oxidative stress-mediated gastric damage (Abdeen et al. 2020). Excessive consumption of NSAIDs is believed to be linked with 20-30% of gastric ulcer cases (García-Rayado et al. 2018). Various studies have reported the effectiveness of medicinal plants against NSAIDs-induced gastric ulcers (Asnaashari et al. 2018). Therefore, this study

searched for the preventive effects of SME against PRX-induced gastric lesions and compared the effects with OMP.

In this study, PRX significantly reduced the gastric pH and increased the acid volume along with gastric acidity (free and total), as observed in a previous study (Abdeen et al. 2019). The SME treated groups demonstrated a significant elevation in gastric acid pH and a reduction in gastric acid volume and acidity comparable to OMP treated group. Also, the ulcer score and ulcer index were markedly decreased in groups administered with SME or OMP when compared to PRX alone treated rats. Histopathological analysis of SME or OMP-treated rats showed ameliorated effects on gastric tissue lesions and disruption of mucosal lining with hemorrhage caused by PRX administration. The SME treatment inhibited gastric acid secretion, as evidenced by an increase in gastric acid pH and a decrease in gastric acid volume and acidity (free and total) in a dose-dependent manner. This study also suggests that SME prevented the development of gastric ulceration might be due to its potential to inhibit histamine-mediated gastric acid secretion induced by PRX (Abdeen et al. 2020). It also might be due to the diminishing inhibitory effect of PRX on prostaglandin synthesis, subsequently normalizing the blood circulation towards the gastric mucosa (Ogaly et al. 2021).

The qualitative as well as quantitative phytochemical characterization of SME revealed that it contains flavonoids, phenols, alkaloids, glycosides, tannins, quinones, lipids, proteins, and carbohydrates. Flavonoids present in SME may prevent gastric mucosal injury by inhibiting lipid peroxidation and enhancing the protective mucosal secretions through prostaglandins synthesis (Khan et al. 2017). The SME lowered gastric acid secretion may be due to the presence of tannins. Tannin tends to precipitate the gastric mucosal proteins exposed at the ulcer site and form a protective lining that prevents gastric mucosal irritation. It also has the ability to decrease gastric acid secretion by inhibiting H^+K^+ ATPase as it actively binds at the ATP hydrolysis site (Eriyamremu and Iorliam 2018; de Veras et al. 2021). These findings are also corroborated by the results of gastric histopathological examination of rats treated with SME or OMP plus PRX, which showed improved gastric histoarchitecture in comparison to positive control group.

PRX is linked with gastrointestinal tract damage and consequently causes acute or chronic bleeding. In this study, PRX administration resulted in significantly lower RBC count and Hb concentration when compared to normal rats, as reported by Abdeen et al. (2019). It could indicate the induction of anemia and a reduction in the oxygen-carrying capacity of blood. The SME treatment markedly restored the RBC count and Hb concentration to the normal level, which suggests that SME has the potential to protect against PRX-induced hematotoxicity by restoring RBCs and enhancing Hb concentration. In the PRX-alone treated group, WBCs and PLTs increased significantly more than in normal control group, indicating compromised host immunity (Alexandrova et al. 2007). Treatment with SME restored the WBC to normal, and it may be attributed to the markedly enhanced host's immune defense system. A significant change in the PLT count of PRX-treated group was found, suggesting its ability to activate the blood clotting mechanism *via* stimulating thrombopoietin production (Ayaz et al. 2017; Nwangwa et al. 2018).

A constant balance between ROS production and scavenging potential is essential for the integrity and normal function of cells or tissues. However, an imbalance among them causes oxidative stress, which disrupts normal cellular functions (Abdeen et al. 2020). In the present study, a significant increase in TOS and MDA levels as well as a decrease in TAC level and CAT activity in serum of PRX-ulcerated rats is an indication of excessive generation of ROS. Over-production of ROS and reduced antioxidant activities could be important factors in PRX-induced toxicity. NSAIDs have been shown in animal models to inhibit antioxidant enzyme activity, resulting in oxidative gastric injury (de Almeida et al., 2017; Meng et al., 2019). However, SME treatment demonstrated a significant decrease in TOS and MDA levels, accompanied by a marked increase in TAC level and CAT activity, indicating the potential antioxidant activity of SME. Our results are consistent with the earlier studies that reported the antioxidant and anti-inflammatory effects of different plants against NSAIDs-induced gastric ulcers (Berezi et al. 2017).

Previous research has found that SME has antioxidant properties (Olanike and Adebawale, 2011; Rogowska et al., 2015; Rogowska et al., 2017). This antioxidant effect of SME on gastric mucosa suggests its gastroprotective effect against PRX-induced gastric ulcers. Phytoconstituents detected in SME might have prevented gastric ulcers through different mechanisms, along with the antioxidant activity. Taken together, our study findings suggested that SME protected against the damaging effects of PRX through stimulation of antioxidant enzymes and inhibition of lipid peroxidation. In addition, SME still requires phytochemical characterization to fully elucidate its composition, and the possibility of gastroprotective activity of a single or combined phytochemical through different mechanisms cannot be excluded, which requires further studies.

Conclusion

This study's findings indicate that administration of *S. media* extract attenuated piroxicam-induced gastric damage, normalized the altered hematological parameters and ameliorated histopathological abrasions in rat's stomach. The antioxidant activity of *S. media*, which contains phytoconstituents detected by HPLC and FTIR, may be responsible for its gastroprotective effect. Overall, the findings supported the effectiveness of *S. media* in preventing drug-induced gastric damage, suggesting that it could be used as an alternative approach to treating gastric ulcers.

Abbreviations

AA: Ascorbic acid; CAT: Catalase; CE: Catechin equivalent; DPPH: 1,1-diphenyl-2-picrylhydrazyl; FT-IR: Fourier transform-infrared spectroscopy; GAE: Gallic acid equivalent; HPLC: High performance liquid chromatography; MDA: Malondialdehyde; OMP: Omeprazole; PRX: piroxicam; TAC: Total antioxidant capacity; TOS: Total oxidant status; TFC: Total flavonoid content; TPC: Total phenolic content; SME: *S. media* methanolic extract

Declarations

Funding: This study did not receive any financial support.

Competing interests: All authors have declared no competing interests.

Consent to participate: Not applicable

Consent for publication: Not applicable

Ethical approval: The experimental protocols were approved by the Institutional Bioethical Committee (IBC), UAF (D. No. 2006/ORIC). The NIH guidelines (No. 85-23, 2002) were implemented throughout the experiment.

Availability of data and materials: The dataset used and analyzed during the current study are available as supplementary material.

Authors' contributions: Study design: AA, BA, and MNF. Conducted study and collected data: AA, MBA, AH, and WM. Interpretation of results: BA, MNF, and WM. Manuscript-writing: AA, MBA, and AH. Manuscript-proof reading: BA and MNF.

Acknowledgments: Not applicable

References

- Abd-Alla HI, Ibrahim Fouad G, Ahmed KA, Shaker K (2022) Alloimperatorin from *Ammi majus* fruits mitigates piroxicam-provoked gastric ulcer and hepatorenal toxicity in rats via suppressing oxidative stress and apoptosis. *Biomarkers* 27(8):727-742. <https://doi.org/10.1080/1354750X.2022.2102213>
- Abdeen A, Abdelkader A, Elgazzar D, Aboubakr M, Abdulah OA, Shoghy K, Abdel-Daim M, El-Serehy HA, Najda A, El-Mleeh A (2020) Coenzyme Q10 supplementation mitigates piroxicam-induced oxidative injury and apoptotic pathways in the stomach, liver and kidney. *Biomed Pharmacother* 130:e110627. <https://doi.org/10.1016/j.biopha.2020.110627>
- Abdeen A, Aboubakr M, Elgazzar D, Abdo M, Abdelkader A, Ibrahim S, Elkomy A (2019) Rosuvastatin attenuates piroxicam-mediated gastric ulceration and hepato-renal toxicity in rats. *Biomed Pharmacother* 110:895-905. <https://doi.org/10.1016/j.biopha.2018.11.004>
- Adefisayo MA, Akomolafe RO, Akinsomisoye SO, Alabi QK, Ogundipe OL, Omole JG, Olamilosoye KP (2017) Gastro-protective effect of methanol extract of *Vernonia amygdalina* (del.) leaf on aspirin-induced gastric ulcer in Wistar rats. *Toxicol Rep* 4:625-633. <https://doi.org/10.1016/j.toxrep.2017.11.004>
- Ahmad W, Ahmad M, Sahibzada MU, Khusro A, Emran TB, Alnasrawi AM, Alkahtani J, Elshikh MS (2022) Lipid peroxidation reduction and hippocampal and cortical neurons protection against ischemic damage in animal model using *Stellaria media*. *Saudi J Biol Sci* 29(3):1887-1892. <https://doi.org/10.1016/j.sjbs.2021.10.033>

- Ajuru MG, Williams LF, Ajuru G (2017) Qualitative and quantitative phytochemical screening of some plants used in ethnomedicine in the Niger Delta region of Nigeria. *J Food Nutr Sci* 5(5):198-205. <https://doi.org/10.11648/j.jfns.20170505.16>
- Akhtar AH, Kamal UA (1995) Anti-ulcerogenic evaluation of the methanolic extracts of some indigenous medicinal plants in aspirin-ulcerated rats. *J Ethnopharmacol* 46(1):1-6. [https://doi.org/10.1016/0378-8741\(94\)01220-t](https://doi.org/10.1016/0378-8741(94)01220-t)
- Alexandrova ML, Bochev PG, Markova VI (2007) Inhibitory and enhancing effects of piroxicam on whole blood chemiluminescence. *Luminescence* 22(2):97-104. <https://doi.org/10.1002/bio.932>
- Asnaashari S, Dastmalchi S, Javadzadeh Y (2018) Gastroprotective effects of herbal medicines (roots). *Int J Food Prop* 21(1):902-920. <https://doi.org/10.1080/10942912.2018.1473876>
- Ayaz M, Junaid M, Ullah F, Sadiq A, Shahid M, Ahmad W, Ullah I, Ahmad A, Syed NI (2017) GC-MS analysis and gastroprotective evaluations of crude extracts, isolated saponins, and essential oil from *Polygonum hydropiper* L. *Front Chem* 5:58. <https://doi.org/10.3389/fchem.2017.00058>
- Berezi EP, Uwakwe AA, Monago-Ighorodje CC, Nwauche KT (2017) Gastroprotective potentials of aqueous leaf extracts of *Phyllanthus amarus* on ibuprofen-induced ulcer in Wistar rats. *Int J Adv Res Biol Sci* 4(10):138-146. <https://doi.org/10.22192/ijarbs.2017.04.10.018>
- de Almeida CL, Brito SA, de Santana TI, Costa HB, de Carvalho Júnior CH, da Silva MV, de Almeida LL, Rolim LA, Dos Santos VL, Wanderley AG, da Silva TG (2017) *Spondias purpurea* L. (Anacardiaceae): antioxidant and antiulcer activities of the leaf hexane extract. *Oxid Med Cell Longev* 14:6593073. <https://doi.org/10.1155/2017/6593073>
- de Veras BO, da Silva MV, Ribeiro PPC (2021) Tannic acid is a gastroprotective that regulates inflammation and oxidative stress. *Food Chem Toxicol* 156:112482. <https://doi.org/10.1016/j.fct.2021.112482>
- Demján V, Sója A, Kiss T, Fejes A, Gausz FD, Szűcs G, Siska A, Földesi I, Tengölics R, Darula Z, Csupor D (2022) *Stellaria media* tea protects against diabetes-induced cardiac dysfunction in rats without affecting glucose tolerance. *J Tradit Complement Med* 12(3):250-259. <https://doi.org/10.1016/j.jtcme.2021.08.003>
- Erel O (2004) A novel automated method to measure total antioxidant response against potent free radical reactions. *Clin Biochem* 37(2):112-119. <https://doi.org/10.1016/j.clinbiochem.2003.10.014>
- Erel O (2005) A new automated colorimetric method for measuring total oxidant status. *Clin Biochem* 38(1):1103-1111. <https://doi.org/10.1016/j.clinbiochem.2005.08.008>
- Eriyamremu GE, Iorliam EB (2018) Gastroprotective effect of tannin from *Schwenkia americana* against aspirin-induced gastric ulcer. *Int J Med Biomed Res* 7(1):32-39.

- García-Rayado G, Navarro M, Lanas A (2018) NSAID induced gastrointestinal damage and designing GI-sparing NSAIDs. *Expert Rev Clin Pharmacol* 11(10):1031-1043. <https://doi.org/10.1080/17512433.2018.1516143>
- Goth L (1991) A simple method for determination of serum catalase activity and revision of reference range. *Clin Chim Acta* 196(2–3):143-151. [https://doi.org/10.1016/0009-8981\(91\)90067-m](https://doi.org/10.1016/0009-8981(91)90067-m)
- Gupta M, Kapoor B, Gupta R, Singh N (2021) Plants and phytochemicals for treatment of peptic ulcer: An overview. *S Afr J Bot* 138:105-114. <https://doi.org/10.1016/j.sajb.2020.11.030>
- Hussain A, Aslam B, Muhammad F, Faisal MN, Kousar S, Mushtaq A, Bari MU (2021) Anti-arthritic activity of *Ricinus communis* L. and *Withania somnifera* L. extracts in adjuvant-induced arthritic rats via modulating inflammatory mediators and subsiding oxidative stress. *Iran J Basic Med Sci* 24(7):951-961. <https://doi.org/10.22038/ijbms.2021.55145.12355>
- Kamada T, Satoh K, Itoh T, Ito M, Iwamoto J, Okimoto T, Kanno T, Sugimoto M, Chiba T, Nomura S, Mieda M (2021) Evidence-based clinical practice guidelines for peptic ulcer disease 2020. *J Gastroenterol* 56(4):303-322. <https://doi.org/10.1007/s00535-021-01769-0>
- Kavitt RT, Lipowska AM, Anyane-Yeboah A, Gralnek IM (2019) Diagnosis and treatment of peptic ulcer disease. *Am J Med* 132(4):447-456. <https://doi.org/10.1016/j.amjmed.2018.12.009>
- Khan MSA, Nazan S, Jais AMM (2017) Flavonoids and anti-oxidant activity mediated gastroprotective action of leathery murdah, *Terminalia coriacea* (roxb.) wight & arn. leaf methanolic extract in rats. *Arch Gastroenterol* 54(3):183-191. <https://doi.org/10.1590/S0004-2803.201700000-21>
- Khan R, Ahmad W, Ahmad M (2019) *Stellaria media* attenuates the hyperglycemia and hyperlipidemia in alloxan-induced diabetic rat. *Bangladesh J Pharmacol* 14(2):80-86. <https://doi.org/10.3329/bjp.v14i2.39847>
- Kuna L, Jakab J, Smolic R, Raguz-Lucic N, Vcev A, Smolic M (2019) Peptic ulcer disease: a brief review of conventional therapy and herbal treatment options. *J Clin Med* 8(2):179. <https://doi.org/10.3390/jcm8020179>
- Lanas A, Chan FKL (2017) Peptic ulcer disease. *Lancet* 390(10094):613-624. [https://doi.org/10.1016/S0140-6736\(16\)32404-7](https://doi.org/10.1016/S0140-6736(16)32404-7)
- Meng J, Chen T, Zhao Y, Lu S, Yu H, Chang Y, Chen D (2019) Study of the mechanism of anti-ulcer effects of virgin coconut oil on gastric ulcer-induced rat model. *Arch Med Sci* 15(5):1329-1335. <https://doi.org/10.5114/aoms.2018.76943>
- Nwangwa EK, Anachuna KM, Ekhoje EI, Chijiokwu-Agbonifo E (2018) Deteriorating hemostatic functions of adult female Wistar rats mediated by activities of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)– piroxicam and Vitamin E. *Niger J Physiol Sci* 33(1):69-73.

- Ogaly HA, Alsherbiny MA, El Badawy SA, Abd-Elsalam RM, Li CG, Azouz AA (2021) Gastroprotective effects and metabolomic profiling of Chasteberry fruits against indomethacin-induced gastric injury in rats. *J Funct Foods* 86(1):104732. <https://doi.org/10.1016/j.jff.2021.104732>
- Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 95(2):351-358. [https://doi.org/10.1016/0003-2697\(79\)90738-3](https://doi.org/10.1016/0003-2697(79)90738-3)
- Oladeji OS, Oyebamiji AK (2020) *Stellaria media* (L.) Vill.-A plant with immense therapeutic potentials: phytochemistry and pharmacology. *Heliyon* 6(6):e04150. <https://doi.org/10.1016/j.heliyon.2020.e04150>
- Olanike B, Adebawale B (2011) Phytochemistry and *in vitro* anti-oxidant activities of *Stellaria media*, *Cajanus cajan* and *Tetracera potatoria* methanolic extracts. *J Med Plant Res* 5(30):6622-6627. <https://doi.org/10.5897/JMPR11.246>
- Oyebanji BO, Saba AB, Oridupa OA (2012) Anti-inflammatory and analgesic effects of methanol extract of *Stellaria media* (L.) Vill leaf. *Afr J Biomed Res* 15(1):29-34.
- Peskar BM, Ehrlich K, Peskar BA (2002) Role of ATP-sensitive potassium channels in prostaglandin-mediated gastroprotection in the rat. *J Pharmacol Exp Ther* 301:969-974. <https://doi.org/10.1124/jpet.301.3.969>
- Ray A, Gulati K, Henke P (2020) Stress gastric ulcers and cytoprotective strategies: perspectives and trends. *Curr Pharm Des* 26:2982-2990. <https://doi.org/10.2174/1381612826666200521143203>
- Rogowska M, Lenart M, Srećec S, Ziaja M, Parzonko A, Bazyłko A (2017) Chemical composition, antioxidative and enzyme inhibition activities of chickweed herb (*Stellaria media* L., Vill.) ethanolic and aqueous extracts. *Ind Crops Prod* 97:448-454. <https://doi.org/10.1016/j.indcrop.2016.12.058>
- Rogowska M, Parzonko A, Bazyłko A (2015) *In vitro* antioxidant and anti-inflammatory activity, and determination of chemical composition of aqueous and ethanolic extracts of *Stellaria media* herb. *Planta Med* 81:16. <https://doi.org/10.1055/s-0035-1565659>
- Saganuwan SA (2017) Piroxicam: Source for synthesis of central nervous system (CNS) acting drugs. *Cent Nerv Syst Agents Med Chem* 17:135-140. <https://doi.org/10.2174/1871524916666160719102335>
- Sattar A, Abdo A, Mushtaq MN, Anjum I, Anjum A (2019) Evaluation of gastro-protective activity of *Myristica fragrans* on ethanol-induced ulcer in albino rats. *An Acad Bras Cienc* 91(2):e20181044 <http://dx.doi.org/10.1590/0001-3765201920181044>
- Seal T (2016) Quantitative HPLC analysis of phenolic acids, flavonoids and ascorbic acid in four different solvent extracts of two wild edible leaves, *Sonchus arvensis* and *Oenanthe linearis* of North-Eastern region in India. *J Appl Pharm Sci* 6(2):157-166. <https://doi.org/10.7324/JAPS.2016.60225>

Subhan F, Aslam B, Muhammad F, Faisal MN, Hussain A, Bari MU (2021) The efficiency of *Cressa cretica* (Linn.) extract in attenuating vancomycin-induced nephrotoxicity and oxidative stress in rabbits. *Comp Clin Path* 30:829-839. <https://doi.org/10.1007/s00580-021-03263-8>

Sultana B, Anwar F, Ashraf M (2009) Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules* 14:2167-2180. <https://doi.org/10.3390/molecules14062167>

Teuşdea AC, Laslo V, Cavalu S, Fritea L, Dobjanschi L, Zdrinca M, Zdrinca M, Ganea M, Paşc P, Memete AR, Antonescu A (2021) Evaluation of *in vitro* wound-healing potential, antioxidant capacity, and antimicrobial activity of *Stellaria media* (L.) Vill. *Appl Sci* 11(23):11526. <https://doi.org/10.3390/app112311526>

Zhang QW, Lin LG, Ye WC (2018) Techniques for extraction and isolation of natural products: A comprehensive review. *Chin Med* 13:1-26. <https://doi.org/10.1186/s13020-018-0177-x>

Tables

Table 1 Retention times and concentrations of phytochemicals detected in SME

RT (min)	Phytochemicals	Area (mV.s)	Concentration (mg/g)
2.910	Chlorogenic acid	589,546.9	76.64
3.267	Gallic acid	861,904.3	75.84
6.784	Hydroxybutyric (HB) acid	59,856.4	9.58
7.994	Vanillic acid	68,617.0	5.33
11.590	Kaempferol	341,065.4	13.91
15.353	Salicylic acid	110,439.9	41.64
16.032	Coumarin	92,755.0	111.31
24.138	Rutin	34,906.4	3.94

RT: Retention time

Table 2 Functional groups and phytochemicals identified in SME

Wave number (cm ⁻¹)	Functional group	Phytochemicals identified
3356	O-H stretching, H-bonded	Alcohols, phenols (hydroxyl group)
2922	Asymmetric stretching of - CH(CH ₂) vibration	Lipids (saturated aliphatic compounds)
2854	Symmetric stretching of -CH(CH ₂) vibration	Fatty acid and lipids
1711	C=O stretching	Flavonoids, lipids (carbonyl groups)
1453	C=C, C-H stretching	Flavonoids, Aromatic compound
1376	O-H bending	Phenols and/or tertiary alcohols (alcoholic group)
1158	C-OH bending, C-O stretching	Alcohols, Lipids
609	C-I, C-Cl stretching	Halogen compounds, alkyl halides (iodo-compound, chloro-compound)

Table 3 Effect of SME on gastric acid secretion of PRX-induced gastric ulcers in rats

Groups	Gastric acid volume (mL)	Gastric pH	Free acidity (mEq/L/24 h)	Total acidity (mEq/L/24 h)
Normal control	2.07 ± 0.23 ^c	3.89 ± 0.08 ^a	4.87 ± 0.46 ^c	7.42 ± 0.34 ^{cd}
Positive control	4.38 ± 0.19 ^a	2.27 ± 0.12 ^c	9.64 ± 0.44 ^a	12.15 ± 0.40 ^a
PRX+OMP (20 mg/kg)	2.04 ± 0.14 ^c	4.19 ± 0.18 ^a	4.41 ± 0.37 ^c	6.47 ± 0.34 ^d
PRX+SME (150 mg/kg)	3.37 ± 0.16 ^b	3.23 ± 0.11 ^b	6.78 ± 0.31 ^b	10.33 ± 0.58 ^b
PRX+SME (300 mg/kg)	2.41 ± 0.21 ^c	3.82 ± 0.09 ^a	5.26 ± 0.23 ^c	8.29 ± 0.35 ^c
PRX+SME (450 mg/kg)	2.38 ± 0.15 ^c	3.96 ± 0.18 ^a	4.99 ± 0.29 ^c	7.71 ± 0.46 ^c

Results mention as mean ± SEM, n = 6. Different superscripts (a-d) indicate significant (P < 0.05) differences between groups. OMP: omeprazole, PRX: piroxicam, SME: methanolic extract of *S. media*

Table 4 Effect of SME on gastric ulcer scoring parameters of PRX-induced gastric ulcers in rats

Groups	Ulcer score	Ulcer index	Ulcer inhibition
Normal control	---	---	---
Positive control	5.67 ± 0.33 ^a	5.29 ± 0.30 ^a	---
PRX+OMP (20 mg/kg)	1.50 ± 0.27 ^d	1.36 ± 0.11 ^d	72.02 ± 1.78 ^a
PRX+SME (150 mg/kg)	4.61 ± 0.42 ^b	4.38 ± 0.31 ^b	20.86 ± 1.46 ^c
PRX+SME (300 mg/kg)	2.50 ± 0.22 ^c	2.32 ± 0.22 ^c	59.25 ± 1.18 ^b
PRX+SME (450 mg/kg)	1.83 ± 0.31 ^{cd}	1.57 ± 0.19 ^{cd}	68.48 ± 1.54 ^a

Results mention as mean ± SEM, n = 6. Different superscripts (a-d) indicate significant (P < 0.05) differences between groups. OMP: omeprazole, PRX: piroxicam, SME: methanolic extract of *S. media*

Table 5 Effect of SME on hematological indices of PRX-induced gastric ulcers in rats

Groups	RBCs (10 ⁶ /μL)	Hb (g/dL)	WBCs (10 ³ /μL)	PLTs (10 ³ /μL)
Normal control	6.74 ± 0.21 ^a	14.12 ± 0.48 ^a	4.88 ± 0.29 ^{bc}	556.17 ± 24.01 ^{ab}
Positive control	5.36 ± 0.27 ^b	12.27 ± 0.36 ^b	7.11 ± 0.25 ^a	504.68 ± 23.92 ^b
PRX+OMP (20 mg/kg)	6.10 ± 0.32 ^{ab}	13.16 ± 0.39 ^{ab}	5.69 ± 0.30 ^b	543.11 ± 28.22 ^{ab}
PRX+SME (150 mg/kg)	6.23 ± 0.29 ^a	13.09 ± 0.38 ^{ab}	4.98 ± 0.27 ^{bc}	562.56 ± 24.41 ^{ab}
PRX+SME (300 mg/kg)	6.56 ± 0.27 ^a	13.90 ± 0.33 ^a	4.70 ± 0.26 ^c	599.74 ± 21.74 ^a
PRX+SME (450 mg/kg)	6.78 ± 0.28 ^a	13.98 ± 0.34 ^a	4.56 ± 0.31 ^c	610.15 ± 26.91 ^a

Results mention as mean ± SEM, n = 6. Different superscripts (a-c) indicate significant (P < 0.05) differences between groups. OMP: omeprazole, PRX: piroxicam, SME: methanolic extract of *S. media*

Figures

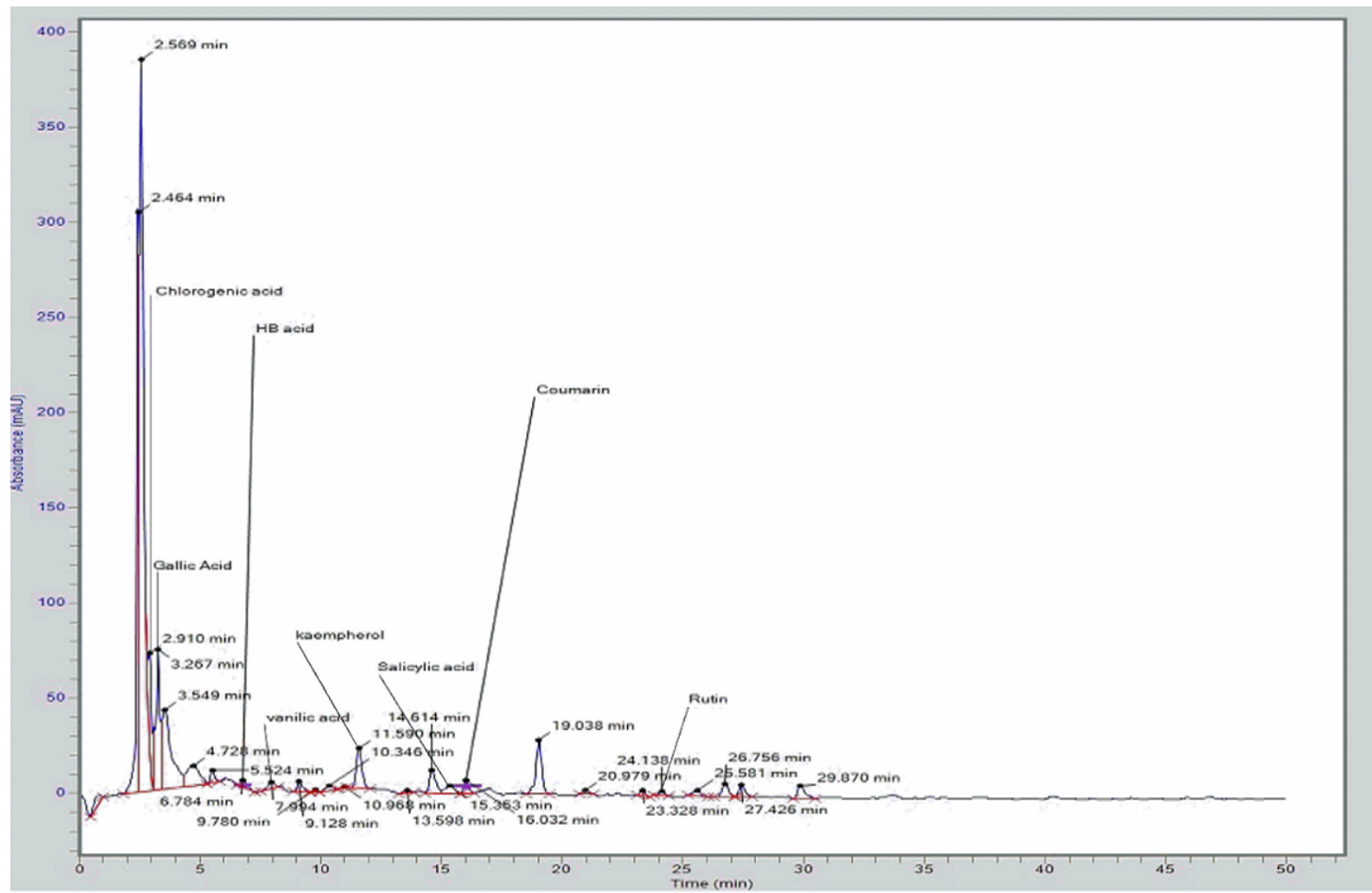


Figure 1

HPLC chromatogram of SME

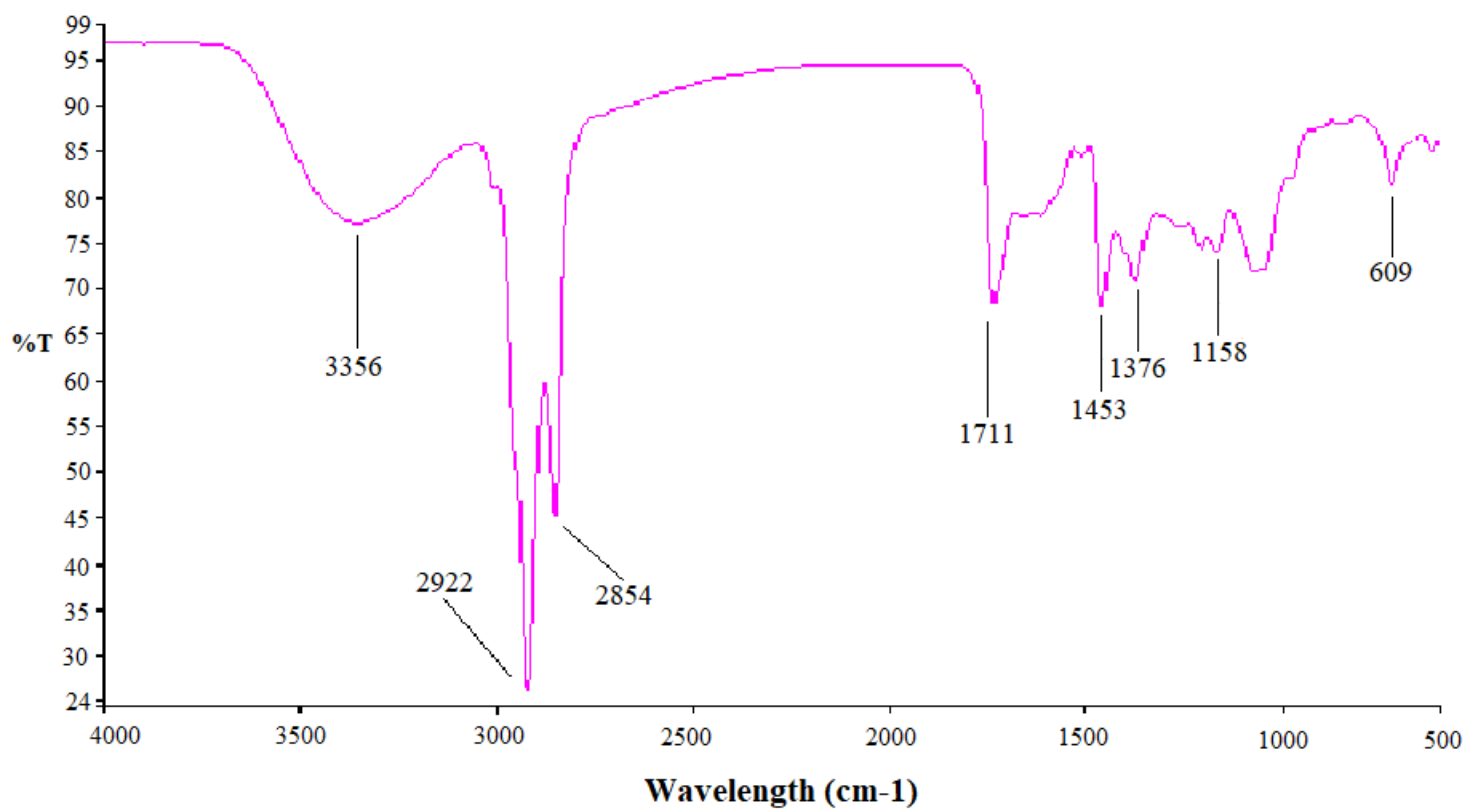


Figure 2

FT-IR spectrum analysis of SME showing different functional groups

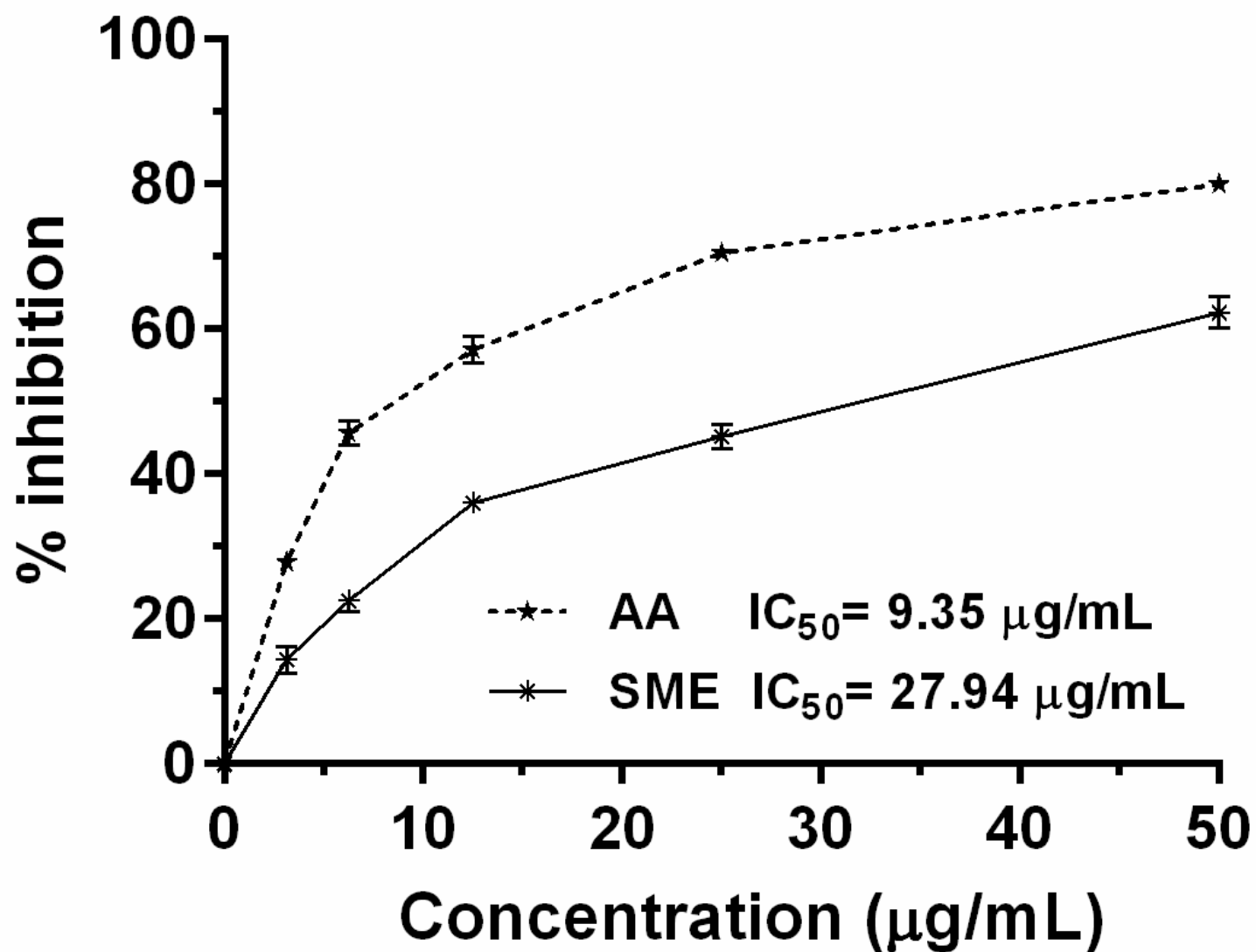


Figure 3

In vitro antioxidant activity of SME in DPPH scavenging assay. Results presented as mean $\pm$ SEM ($n = 3$). AA: ascorbic acid, SME: methanolic extract of *S. media*

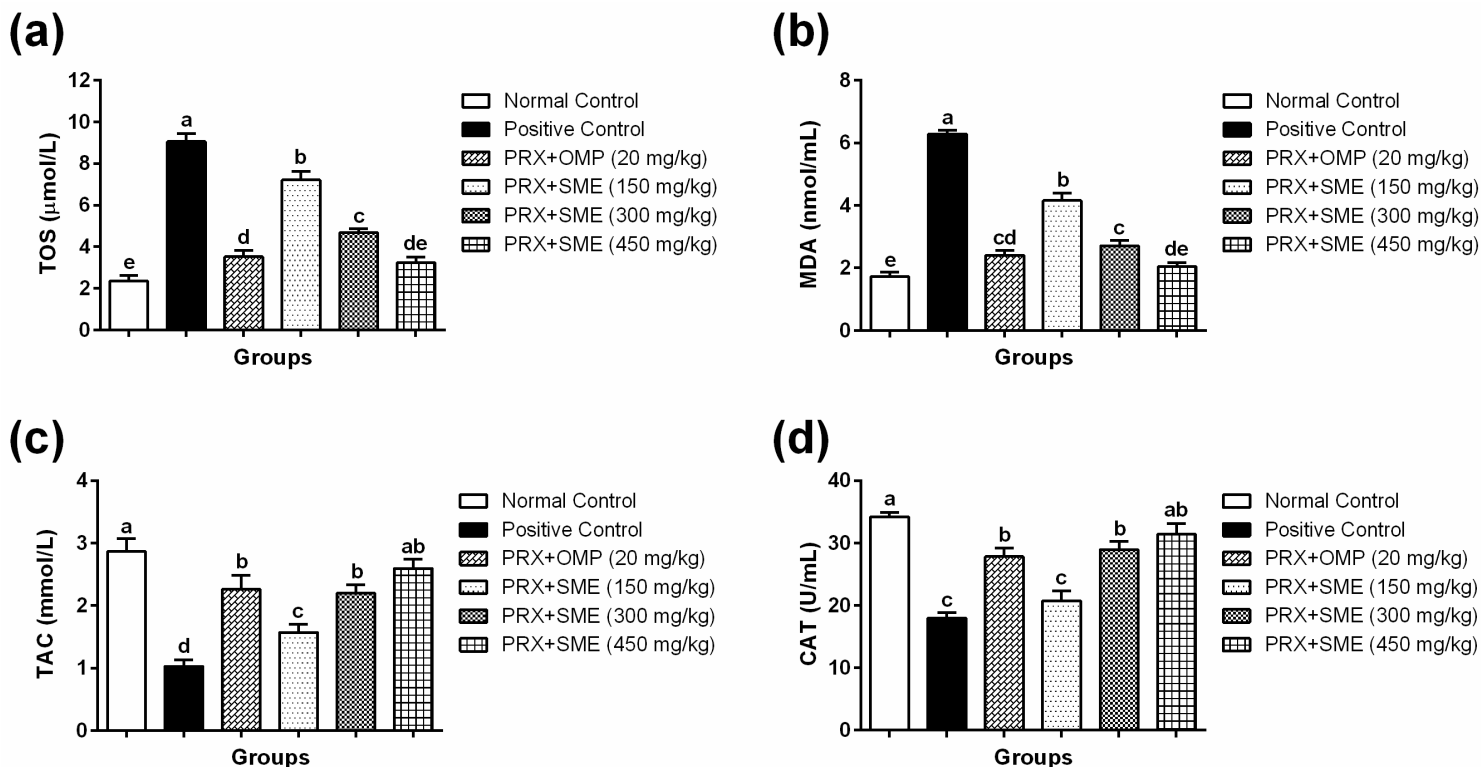


Figure 4

Effect of SME on serum oxidant/antioxidant parameters of PRX-induced gastric ulcers in rats. **(a)** Total oxidant status (TOS) level, **(b)** malondialdehyde (MDA) level, **(c)** total antioxidant capacity (TAC) level, and **(d)** catalase (CAT) activity. Results mention as mean $\pm$ SEM, n = 6. Different superscripts (a-e) indicate significant ($P < 0.05$) differences between groups. OMP: omeprazole, PRX: piroxicam, SME: methanolic extract of *S. media*

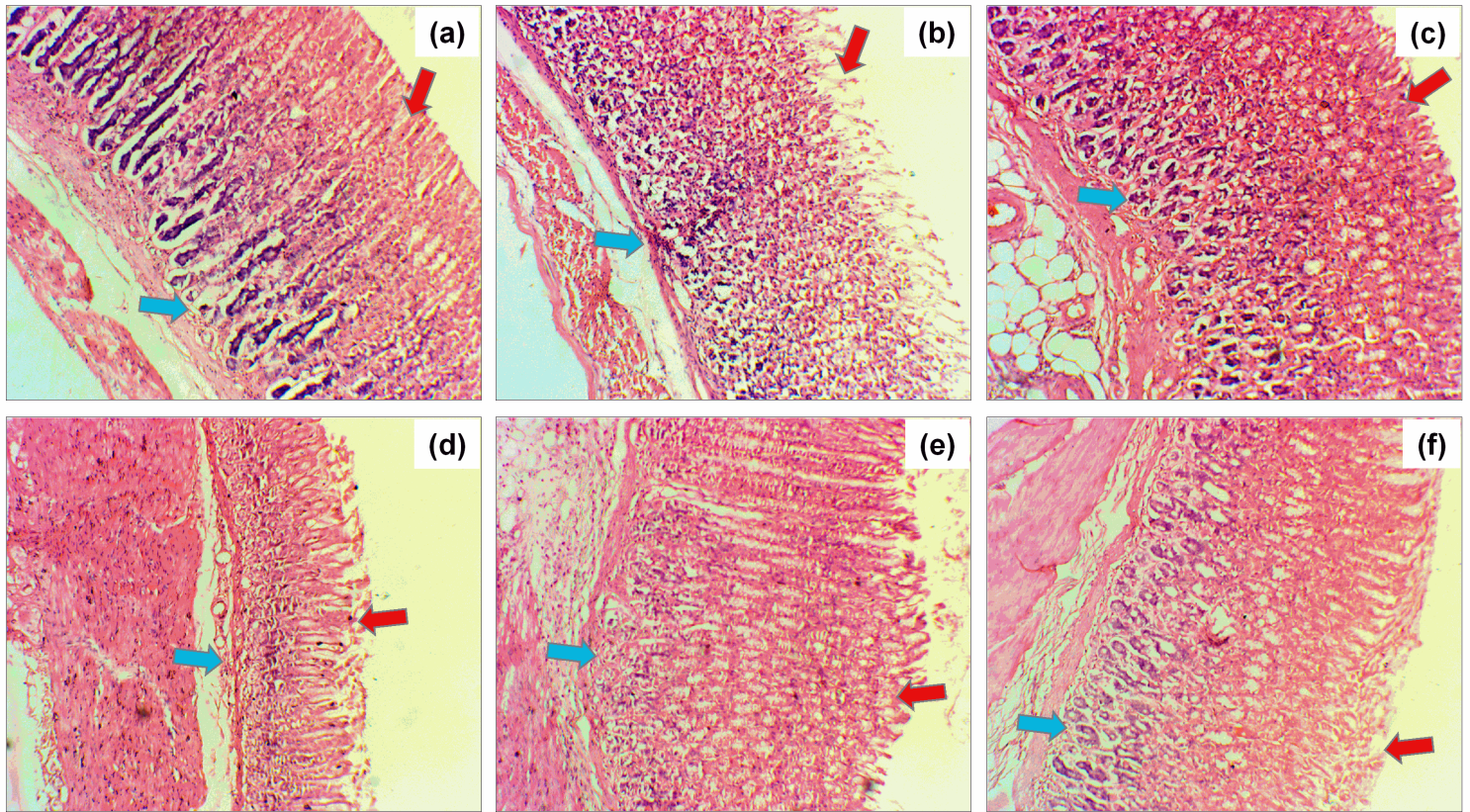


Figure 5

Effect of SME on stomach histology of rats against PRX-induced gastric ulcer (H&E stained; x100 magnification). **(a)** Normal control indicated intact gastric epithelium (red arrow) and submucosal linings (blue arrow). **(b)** Positive control revealed degenerative changes, including disruption of mucosal lining and development of sub epithelial space. **(c)** PRX plus OMP 20 mg/kg showed intact gastric architecture. **(d-f)** SME administration at 150, 300, and 450 mg/kg exhibited dose-dependent protection of gastric histology. OMP: omeprazole, PRX: piroxicam, SME: methanolic extract of *S. media*

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [GraphicalAbstract.tif](#)
- [Supplementarydatafile.pzfx](#)