RESEARCH PAPER

Phytochemical Profiling and Evaluation of the Gastroprotective Activity of Polygonum bistorta Root Extract Using FTIR, HR-LCMS, and an Immobilization Stress-Induced Peptic Ulcer Model in Rats

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Received: 28th Feb, 2026; Revised: 6th March 2026; Accepted: 7th April, 2026; Available Online: 20th April, 2026

ABSTRACT
Psychophysiological stress is increasingly recognized as a critical contributing factor in the pathogenesis of peptic ulcer disease, independent of Helicobacter pylori infection and the use of non-steroidal anti-inflammatory drugs (NSAIDs). The present study aimed to investigate the phytochemical profile and gastroprotective activity of a hydroalcoholic extract of Polygonum bistorta roots using an immobilization stress-induced gastric ulcer model in rats. Phytochemical characterization was performed using high-resolution liquid chromatography–mass spectrometry (HR-LCMS), while Fourier transform infrared (FTIR) spectroscopy was employed to confirm the presence of major bioactive functional groups associated with polyphenols, flavonoids, and tannins. The findings suggest that the extract possesses significant phytoconstituents with potential antioxidant and gastroprotective properties, supporting its traditional therapeutic use in the management of gastric ulceration. Gastric ulcers were induced by immobilization stress, followed by post-treatment with the extract. Gastroprotective activity was further evaluated through the assessment of oxidative stress biomarkers in gastric tissues, including reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA). The analysis of these biochemical parameters provided insight into the antioxidant defense mechanisms and lipid peroxidation status associated with stress-induced gastric mucosal injury. Histopathological examination was conducted to validate mucosal protection.Treatment with P. bistorta extract significantly reduced ulcer index and lipid peroxidation while restoring antioxidant enzyme levels and gastric mucus content compared to the untreated control group. Histological analysis further confirmed marked preservation of gastric mucosal integrity. The observed effects are likely attributed to the presence of bioactive phytoconstituents identified through HR-LCMS and supported by FTIR analysis.In conclusion, the hydroalcoholic extract of Polygonum bistorta exhibits significant gastroprotective and antioxidant activity in an immobilization stress-induced ulcer model. These findings suggest its potential as a natural therapeutic candidate for stress-related gastric ulcers, warranting further investigation for clinical applications.

Keywords: *Polygonum bistorta, HR-LCMS, FTIR, gastroprotection, immobilization stress, peptic ulcer, oxidative stress*
How to cite this article: Ahmad A, Yadav MK, Mishra A. Phytochemical Profiling and Evaluation of the Gastroprotective Activity of Polygonum bistorta Root Extract Using FTIR, HR-LCMS, and an Immobilization Stress-Induced Peptic Ulcer Model in Rats. Int J Drug Deliv Technol. 2026;16(64s):1240-1256. DOI: 10.25258/ijddt.16.64s.131
Source of support: Nil.
Conflict of interest: None

INTRODUCTION

A considerable percentage of people worldwide suffer from Peptic Ulcer Disease (PUD), a chronic illness .[1,2] The pH of the stomach juice and the weakened mucosal defence have an impact on the development and pathophysiology of PUD. [1,3] The most widely recognized and researched danger PUD-related factors include, among other things, taking NSAIDs (non-steroidal anti-inflammatory drugs) and being exposed to Helicobacter pylori (H. pylori). Ulcers unrelated to NSAIDs or H. pylori have become more common, which has an impact on the choice and effectiveness of traditional treatment regimens for PUD, even though Warren and Marshall were awarded the Nobel Prize in 2005 for demonstrating the role of H. pylori in PUD. [4] Research revealed that a large number of individuals with PUD did not have a history of NSAID use or H. pylori infection. [2, 5] These ulcers are idiopathic and extremely difficult to treat .[6] The large number of H. pylori-positive people who never experienced PUDs highlights the development of PUD without H. pylori. [4,7, 8] On the other hand, although H. pylori infection decreased as a result of improved control measures, PUD incidences continue to rise, making the role of H. pylori in the pathophysiology of PUD less evident. [5, 9] There is strong evidence that the development of these types of ulcers is influenced by psychophysiological stress, and

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there is a strong enough correlation between PUD and stress to warrant consideration. According to a study, between 30% and 65% of PUD cases involve stress.[6, 10] It has been proposed that different stress strategies that are identical to the pathogenesis of human PUDs cause PUD in rodents and other animal models. [11] Oxidative stress, however, has been shown to exacerbate the pathogenesis of PUDs in both human and animal models. [12, 13] Omeprazole is a proton pump inhibitor and a substituted benzimidazole derivative that belongs to the class of antisecretory agents. It exerts its pharmacological effect by irreversibly inhibiting the gastric parietal cell H^+/K^+-ATPase enzyme system, the final step in gastric acid secretion. Consequently, omeprazole suppresses both basal and stimulated gastric acid secretion. [14] Consequently, the pharmacological effects of different herbal formulations have been evaluated, especially with regard to gastrointestinal disorders. [15]

P. bistorta, on the other hand, is one of the most potent acerbics and is used to stop blood flow and contract tissues. [16] P. bistorta's rhizome has strong demulcent, astringent, diuretic, febrifuge, laxative, and potent styptic properties. [17, 19] It is utilized both externally and internally.It treats cholera, diarrhea, dysentery, internal and external bleeding, etc. [20] Tannin compounds (15–22% to 36%) are responsible for the root stock's astringent qualities. The tannin is mixed; it has been identified as phlobaphene, gallic acid, phloroglucinol, and catechols. [21] The plant contains friedelin, β-sitosterol, and cycloartane-type triterpenoids such as 24(E)-ethylidenecycloartanone [22], as well as some tannin-related compounds, such as bistortaside A.(Liu et al., 2006) [23] In the GIT region, polyphenols exhibit a variety of pharmacological characteristics, including antioxidant, cytoprotective, and antisecretory effects. The phenolic compounds' antioxidant qualities have been extensively researched, but it is now evident that their mechanisms of action extend beyond oxidative stress modulation. [15, 24, 25]

The primary class of bioactive compounds responsible for free radical scavenging and antioxidant activity are plant phenolics, including flavonoids and tannins, which act as effective radical scavengers.[26,27] The antioxidant potential of the plant extract and the standard compound was evaluated using a modified method based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.[28] Oxidative stress and gastric injury are known to induce inflammation of the gastric mucosa.[29,30] These effects were further examined in both wild-type and NF-κB p50-deficient mice.[31]

METHODS AND MATERIALS

Reagents or chemicals:
5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) and carboxymethyl cellulose (CMC) were purchased from Sigma-Aldrich (USA) and Fisher Scientific (Navi Mumbai, India), respectively. Alcian blue as well as SD Fine Chem. Ltd. in India provided the trichloroacetic acid (TCA), while H2 O2 was provided by India's Loba Chemie Pvt. Ltd. It was purchased from Sun Pharma Pvt. Ltd. in India.Himedia, India, provided pyrogallol and the remaining analytical-grade chemicals.

Experimentations:

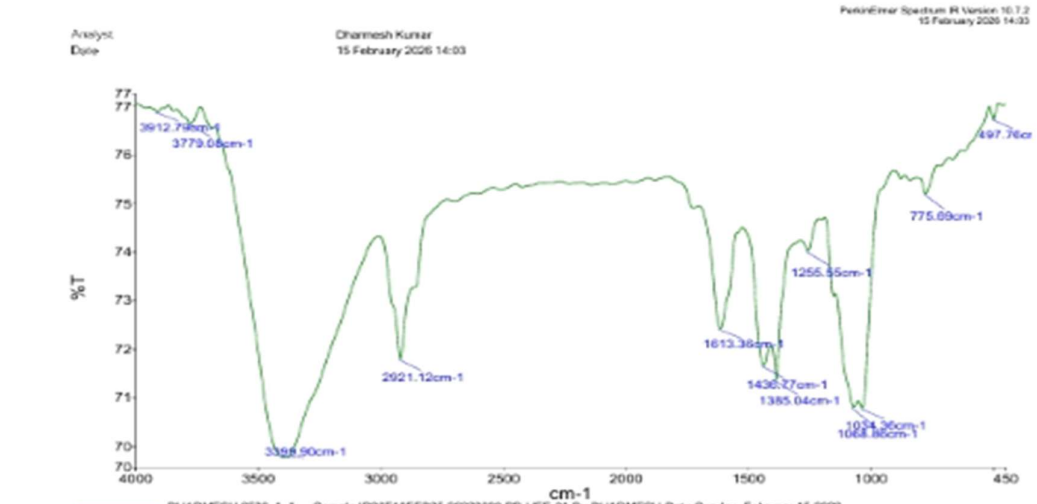
Polygonum bistorta extract preparation;
The plants used in this study were purchased from the neighborhood market, Hamdard Dawakhana, which is situated in Amina Baad Lucknow and was authentication by the herbalist and CSIR-Natonal botanical institute Lucknow,.Polygonum bistorta Linn root, voucher number:358164. First, petroleum ether (60–80°C) was used to defatten the powdered root sections of P. bistorta. After drying in the shade to obtain a dry mass, the defatted marc was extracted using Soxhlet extraction with 70% hydroethanolic ethanol and water. This process was carried out for two daytime at room temperature with sporadic shaking. After filtering,rotavapor was the focus of the solvent (Buchi, USA). After being weighed and its yield percentage (21.95% w/w) determined, the finished extract was kept in a cold location. [32,33,34] Diterpene dimers, including those found in animals: We purchased Wister rats with a weight of 200–250 grammes from the Animal House Facility at Acharya Narendra Dev College of Pharmacy in Babhnan, GondaUnder typical laboratory circumstances, each of the six animals was kept in a polypropylene cage with a 12-hour day/night cycle,22 degrees Celsius, and a relative humidity of a temperature and relative humidity of 50 ± 15%, ± 2°C respectively, and unrestricted access to a standard pellet meal.(kapila pashu ahar Gorakhpur Industries, India) and unlimited drinking water. The animals were separated into experimental and control groups at random. Ethical approval was given by the Institutional Animal Ethical Committee (IAEC), Regd. 1585/Po/Re/S/11/CPCSEA.

Total phenolic contents
Total phenolic content (TPC) of Polygonum bistorta extract was determined using the Folin–Ciocalteu (FC) colorimetric method with slight modifications. [23] Briefly, 1 mL of the extract was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent. After 5 min of incubation, 2.0 mL of 7.5% sodium carbonate (Na_2CO_3) solution was added to the reaction mixture and incubated at room temperature for 30 min. The absorbance was subsequently measured at 765 nm using a Shimadzu UV-1800 UV–Visible spectrophotometer against a reagent blank (Singleton et al., 1999). The explanation of the Polygonum bistorta was measured in mg GAE/g. [35]

Total flavonoid content
Total flavonoid content (TFC) of Polygonum bistorta extract was estimated using the aluminum chloride ($AlCl_3$) colorimetric assay. Briefly, 1 mL of the extract was mixed with distilled water, followed by the addition of 0.3 mL of 5% sodium nitrite ($NaNO_2$). After 5 min, 0.3 mL of 10% aluminum chloride ($AlCl_3$) solution was added to the mixture. Subsequently, 2 mL of 1 M sodium hydroxide

(NaOH) was added, and the final volume was adjusted to 10 mL using distilled water. The absorbance was measured at 510 nm using a UV–Visible spectrophotometer. Total flavonoid content was expressed as milligrams of quercetin equivalents (QE) per gram of extract (mg QE/g extract). [36]

Identification of constituents by IR



FTIR Analysis Discussion

The ethanolic root extract of Polygonum bistorta exhibited a broad absorption band at 3399 cm^{-1} in the FTIR spectrum, which indicated O–H stretching vibrations typical of tannins and phenolic compounds.[37,38] The peak located at 2921 cm^{-1} is indicative of aliphatic C–H stretching.[39] Aromatic C=C stretching, which suggests flavonoid and polyphenolic structures, is confirmed by the absorption band seen at 1613 cm^{-1}.[37,40] Alcohols, phenols, and glycosidic bonds are supported by peaks between 1255 and 1034 cm^{-1}, which are C–O stretching vibrations.[37,41] The band around 775 cm^{-1} provides additional evidence of substituted aromatic rings. These results strongly imply that the extract is rich in polyphenolic compounds, especially flavonoids and tannins, which are in line with phytoconstituents of Polygonum bistorta root that have been previously reported.[42]

Identification of constituents by High Resolution Liquid Chromatography Mass Spectroscopy (HRLCMS) technique:

After considering preliminary phytochemical evaluation, total phenolic content, total flavonoid content and antioxidant study, the selected ethanolic extract was subjected for HRLCMS study. The HRLCMS report produced a compound table which included the list of components (active constituents) present in the sample as follows:

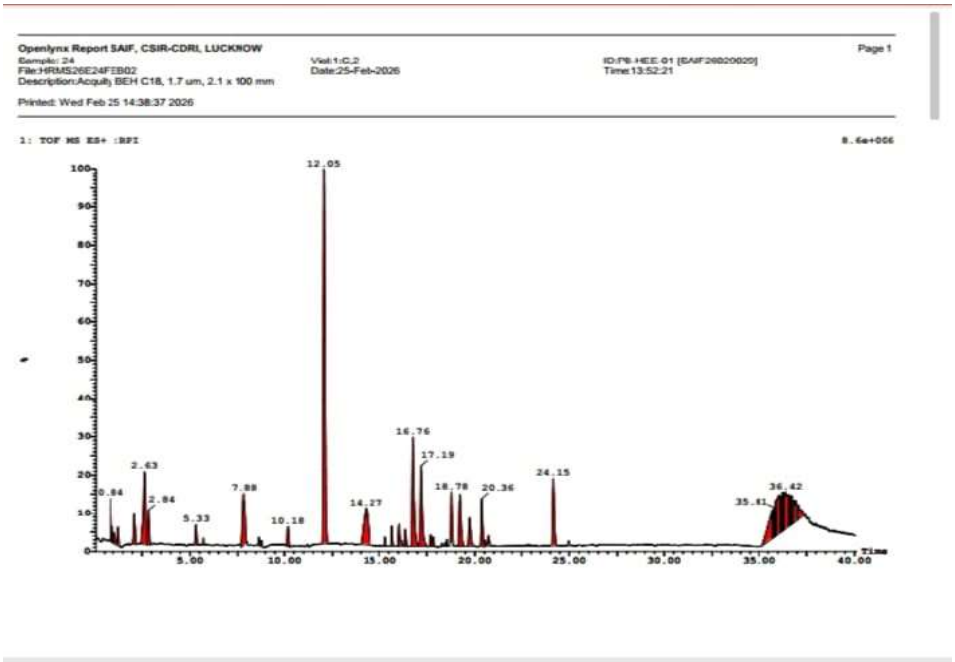


Table X. Tentative identification of major phytoconstituents detected in hydroethanolic extract of Polygonum bistorta root by HR-TOF-MS in positive ionization mode.

Peak ID	RT (min)	Observed m/z ([M+H]⁺)	Calculated Neutral Mass (Da)	Proposed Molecular Formula*	Tentative Identification	Phytochemical Class
11	2.63	355.1033	354.10	C16H18O9 (approx.)	Galloyl glucose derivative	Hydrolysable tannin
15	7.88	453.1196	452.12	C21H20O11 (approx.)	Quercetin-3-O-glucoside	Flavonoid glycoside
20	12.05	326.2317	325.23	C18H29O5 (tentative)	Phenolic conjugate (unconfirmed)	Phenolic derivative
26	16.76	289.2940	288.29	C15H14O6 (approx.)	Catechin	Flavan-3-ol
30	18.78	520.3411	519.34	C30H47O7 (tentative)	Condensed tannin fragment	Proanthocyanidin
39	36.07	289.2382	288.24	C15H14O6 (approx.)	Catechin (isomeric form)	Flavan-3-ol
43	36.42	449.8149	448.81	—	Polyphenolic fragment	Condensed tannin

DISCUSSION OF HR-LCMS

HR-LCMS analysis of the hydroethanolic extract of Polygonum bistorta root revealed a complex phytochemical profile rich in polyphenols, particularly flavonoids and tannins. Detection of multiple ions in positive electrospray ionization mode indicates the presence of structurally diverse secondary metabolites

associated with antioxidant and gastroprotective activities. [43]A prominent ion at m/z 289.29 was tentatively identified as catechin, a flavan-3-ol known for strong antioxidant activity via hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms. Catechin scavenges reactive oxygen species (ROS) and enhances endogenous antioxidant defenses (SOD, CAT, GSH) primarily through activation of the Nrf2–Keap1 signaling pathway, leading to increased expression of antioxidant response element (ARE)-regulated genes. [44] It also contributes to gastro protection by inhibiting lipid peroxidation and enhancing prostaglandin E2-mediated mucus secretion. [43] Another key ion at m/z 453.11 was assigned to quercetin-3-O-glucoside, a flavonoid glycoside with anti-inflammatory and cytoprotective properties. It exerts its effects by suppressing pro-inflammatory mediators (TNF-α, IL-1β, COX-2) via inhibition of the NF-κB signaling pathway. [45] Additionally, it stabilizes mast cells, reduces histamine release, and limits gastric acid secretion, thereby promoting mucosal healing. [45] Peaks in the m/z range of 350–520 suggest the presence of polymeric phenolics and condensed tannins. These compounds contribute to mucosal defense by forming protein–tannin complexes that create a protective barrier over the gastric lining. Their metal-chelating ability further reduces oxidative stress by inhibiting iron-mediated free radical generation. [46] Overall, the extract exhibits a multi-target gastroprotective mechanism involving antioxidant enhancement (Nrf2 activation), anti-inflammatory effects (NF-κB inhibition), increased mucus production, reduced acid secretion, and direct mucosal protection. These findings indicate that the observed anti-ulcer activity is mediated by synergistic interactions of polyphenolic compounds, particularly catechin and quercetin derivatives, supporting the therapeutic potential of Polygonum bistorta root extract. [46]

Selection criteria for the dose of Polygonum bistorta extract: The current study's Polygonum bistorta extract dose was chosen using previously published reports showing up to 2000 mg/kg of Polygonum bistorta extract. In rodents, B.Wt. is safe.These increased Polygonum bistorta dosages demonstrated strong pharmacological effects against various pathologies, and no adverse events were reported in any of these studies. Even such elevated dosages offered protection.the hepatic, intestinal, and sciatic nerve tissues' histoarchitecture.[47,48 ,49 ,50]

Development Of Immobilization Stress-Induced PUD Model

In order to prevent coprophagy, animals were fasted for 24 hours in wire mesh cages with unlimited access to drinking water prior to the disease being induced. The rats were restrained in a supine position using a well-ventilated acryclic restrainer to induce immobilization stress. This limited movement of the limbs and trunk without causing pain or injury. The animals were housed at room temperature and subjected to immobilization stress for a duration of three hours. Animals from Groups I, II, III, and IV were sacrificed by cervical dislocation on days 3, 7, 14, and 21,post induction, respectively. After sacrifice, the stomachs were excised and opened along the greater curvature to assess the development of immobilization stress-induced gastric lesions or ulcers in each group.Upon confirming gastric ulcer induction and subsequent self-healing patterns across Groups I–IV, Group III (sacrificed on day 14) was selected for therapeutic interventional studies, as this group exhibited a significant ulcer index. On the other hand, Group IV (sacrificed on the 21st day) had very small ulcers that might have entirely vanished upon the administration of a standard medication or Polygonum bistorta extract, making it challenging to compare them in the absence of visible ulcers.

Plan for Polygonum bistorta's curative (post-treatment) impact in the immobilization stress-induced PUD model:

After creating a model of gastric ulcers, the immobilization stress test was conducted again to examine the potential therapeutic benefits of Polygonum bistorta extract in combination with omeprazole. A total of twenty-five rats (n=5) were split up into five groups. With the exception of the control group, rats that had fasted for 24 hours were exposed to immobilization stress as described in the preceding section. The rats were taken out of the cylinders and given food and medication. Rats were given 1 ml/kg in Group I (Normal Control (NC)). Group I (Normal Control): Rats were administered 1% CMC orally (dose based on body weight) daily.Group II (Immobilization Stress Control, ISC): Rats were subjected to immobilization stress and sacrificed on the first day after the stress procedure by cervical dislocation.Group III (Self-Healing Control, SHC): Rats were exposed to immobilization stress but did not receive any treatment until the study's conclusion.sGroup IV (Polygonum bistorta Extract Treated, PET): The extract of Polygonum bistorta was administered to rats via gastric intubation at a daily dose of 1000 mg/kg body weight. [51] Group V (Omeprazole Treated, OZT): Omeprazole was administered to rats at a dose of 20 mg/kg body weight per day via oral route. The rats in each group (apart from ISC, which had already been sacrificed) were fasted for 24 hours after the 15-day therapeutic intervention, and they were then sacrificed by cervical dislocation (fig. 1B)

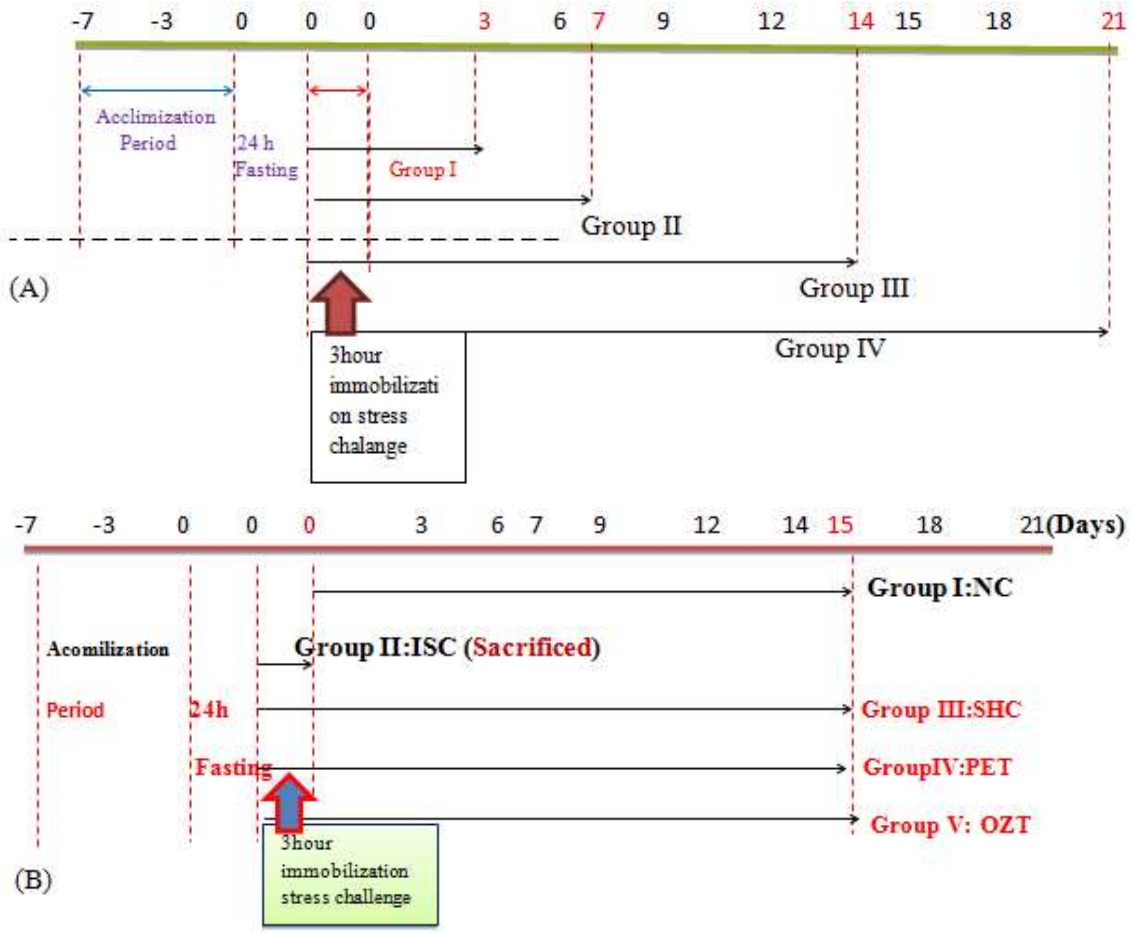


Figure 1: (A) Diagram for creating a rat model of immobilization stress-induced PUD; (B) Diagram for Polygonum bistorta's therapeutic (post-treatment) effect in immobilization stress-induced gastriculcer model

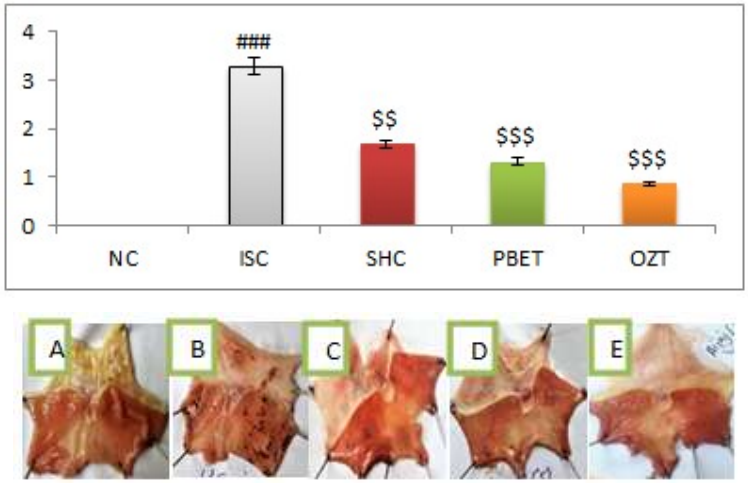


Figure 2: (A) Polygonum bistorta hydroalcoholic extract treats stress-induced gastric ulcers by lowering the ulcer score in a rat model; (B) Polygonum bistorta extract enhances gastric pathomorphology and ulcers in immobilization sstress-induced Model of PUD rats

Note: PET: Polygonum bistorta Extract Treated; ; SHC: Self-Healing Control; OZT treated with omeprazole

ISC: Immobilization Stress Control; NC: Normal Control. ***"*p < 0.001 vs ISC group; ###p < 0.001 vs NC group.

Significantly diferent from ISC rats at $$p<0.01; significantly diferent from SHC rats at**p<0.01;non-signifcantly diferent from PET at nsp>0.05.

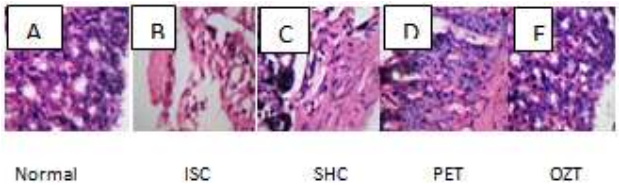


Figure 7: Polygonum bistorta extract enhances gastric histoarchitecture in a rat model of PUD caused by immobilization stress, H&E 40x panels as the staining magnification scale

Gastric tissue collection and processing:
The stomachs of animals from each group were immediately excised after sacrifice, weighed, and rinsed with ice-cold 0.9% NaCl saline solution. The tissues were then stored at −20 °C until further analysis. A portion of each stomach was preserved in 10% neutral buffered formalin for histopathological examination and block preparation..

To create Post Mitochondrial Supernatant (PMS), homogenization and subcellular fractionation are required:
Using a Remi homogenizer (RQ-127-A), sections of the stomach's tissues were homogenized for a variety of biochemical tests in either 0.02 M Ethylenediamine Tetraacetic Acid (EDTA) or chilled PBS. he prepared tissue homogenates were filtered through muslin cloth and subsequently centrifuged at 3000 rpm for 10 minutes to remove cellular and nuclear debris.To obtain PMS, which was necessary for estimating the activities of various enzymatic antioxidants, the resultant aliquots were once more centrifuged at 10,500 xg for 30 minutes.

How the ulcer index is calculated;
Each group's excised stomachs were examined for gastric lesions and indexed as previously mentioned. [11,51] A 10x magnifying glass was used to examine the mucosa after the stomachs were opened and placed on a dissecting tray so that the greater curvature was clearly visible. Most of the lesions developed in the stomach's antrum. The ulcers were counted and scored appropriately. Redness was given a score of 0.5, individual spots were given a score of 1, streak-like bleeding was given a score of 1.5, deep ulcers were given a score of 2, and perforations a score of 3. The ulcer index is calculated using the following formula: The ulcer index (UI) was calculated as follows: UI =UN+US+UP×10-1Where UN is the mean number of ulcers per animal, US is the mean severity score, and UP is the percentage of animals with ulcers.

Calculating the amount of stomach mucus;
Gastric mucus was estimated by the alcian blue binding assay. A glandular portion of the stomach was isolated, washed with ice-cold physiological saline, and stored at −20 °C until use. Tissue samples were incubated in 0.1% alcian blue solution prepared in 50 mM sodium acetate containing 160 mM sucrose (pH 5.8) for 2 h. Excess dye was removed by washing with 250 mM sucrose solution. The dye bound to gastric mucus was then eluted using 500 mM magnesium chloride for 2 h with intermittent shaking. As previously stated, the obtained blue solution was dynamically shaken with (C2 H5)2 O (v/v), and following centrifugation at 3000 rpm, the optical density of the aqueous layer was measured. By creating a calibration curve with different alcian blue concentrations, theassessment of the mucus content .[52]

Calculating lipid peroxide:
The TBA method was used to measure the amount of malondialdehyde (MDA) as reported recently .[53, 54] The malondialdehyde (MDA) level was determined using the thiobarbituric acid reactive substances (TBARS) method as described by Ohkawa et al.[55] Briefly, 500 µL of 0.8% thiobarbituric acid (TBA), 1 mL of tissue homogenate (0.05 M PBS), and 500 µL of trichloroacetic acid (TCA) were mixed and incubated at 80 °C for 30 minutes in the dark. The mixture was then cooled in chilled water and centrifuged at 845.32 × g for 15 minutes. The absorbance of the supernatant was measured at 540 nm using a UV–Visible spectrophotometer. The MDA concentration was calculated using an extinction coefficient of 0.156.

Calculating the amount of glutathione (GSH) in stomach tissue:
A previously established standard method was used to estimate the amount of GSH, a non-enzymatic antioxidant.[56] The stomach tissue was homogenized in a 20 mM EDTA solution, thoroughly vortexed, and combined with 50% TCA.400 mM Tris buffer and 10 mM DTNB were added to the supernatant after centrifugation at 3381.3 xg. The optical density (OD) of the resultant mixture was measured at 415 nm and expressed as µg/mg protein to calculate the amount of GSH.

Calculating the catalase activity:
A previously developed protocol was used to measure the enzymatic antioxidant Catalase (CAT) activity in gastric tissue PMS. [50] In summary, a quartz cuvette containing 2.95 ml of 20 mM H2 O2 was filled with 50 µl of PMS. Catalase (CAT) activity was measured using an Eppendorf UV–VIS spectrophotometer at 240 nm based on the decomposition of hydrogen peroxide (H_2O_2). The decrease in absorbance per minute was recorded. CAT activity was calculated using the formula:CAT Activity= Decline in OD per minute × Reaction volume)/(0.081×homogenate)volume × protein

(mg)).where 0.081 is the extinction coefficient of H_2O_2 at 240 nm, as described by Aebi.

Superoxide dismutase (SOD) activity calculation:
The standard protocol was used to evaluate the SOD activity as part of the CAT [57]. Briefly, 0.1 mL of PMS and 2.9 mL of Tris-HCl buffer were mixed with 25 µL of pyrogallol, and the change in absorbance (OD) was measured at 420nm.Superoxide dismutase (SOD) activity was calculated using the formula:SOD (units/mL sample) = [(A − B) × 100] / (A × 50),where A represents the change in absorbance per minute in the control, and B represents the change in absorbance per minute in the test sample

Analysis of histopathological:
Following the intervention, a portion of the removed stomach was preserved in a 10% neutral buffered formalin solution. Following their embedding in paraffin, hematoxylin and eosin were used to double-stain the blocks after they were sectioned and examined under a microscope at 100x and 400x magnifications. [58, 59]

Examining statistics:
Every experiment was carried out in triplicate, and the mean ± SEM was used to express the results. One-way ANOVA and the Tukey–Kramer post-hoc test were used to assess statistical significance. GraphPad Prism version 4.02 was used to analyze the data. [60, 61]

RESULTS AND DISCUSSION
The development of targeted therapies for peptic ulcer disease (PUD) has been hindered by the lack of appropriate animal models, which are essential for evaluating and validating potential therapeutic agents.[62,63] Pharmacological methods—such as the use of ethanol, acetic acid, histamine, reserpine, and nonsteroidal anti-inflammatory drugs (NSAIDs) like ibuprofen, aspirin, and indomethacin—as well as psychophysiological approaches, represent the most commonly employed experimental models for inducing gastric ulcers.[64] Similarly, a post-treatment immobilization stress-induced rat model of peptic ulcer disease (PUD) was developed by confining animals in vertical cylinders for three hours. Following the induction and subsequent self-healing phase, the stomachs of sacrificed rats were examined for the presence of lesions or ulcers induced by immobilization stress. Gross macroscopic examination revealed that rats in all four groups developed severe gastric ulcers (data not shown).We selected group III (sacrificed on the fourteenth day) to evaluate the therapeutic effect of Polygonum bistorta extract after examining self-healing because Even after the fourteenth day of self-healing, lesions were still easily visible in this group.

Compared to NC rats, the ISC group's gastric ulcer score increased significantly ($p<0.001$) from 0.4±0.02 to 37.12±0.21 following a 3-hour immobilization stress. When left untreated following the immobilization stress challenge, the SHC group's observations showed that the ulcer index decreased to 18.4±0.75 at the conclusion of the examine. However, compared to the corresponding ISC and SHC groups, the PET group's ulcer score decreased by 64.98% and 25.36%, respectively, after receiving a hydro-alcoholic extract of Polygonum bistorta for 15 days ($p<0.001$). These findings indicate that treating immobilization stress-challenged SHC rats with Polygonum bistorta extract promotes a quick recovery. In comparison to the ulcer score in the ISC and SHC groups, the gastric ulcer score was importantly ($p<0.001$) improved by the reference standard drug (OZT) treatment by 63.88% and 25%, respectively (fig. 2).

Our findings indicated that the mucus content was significantly (p0.05), indicating that PET has a therapeutic effect on peptic ulcers brought on by immobilization stress. Additionally, when compared to the ISC and SHC groups, the OZTT group's mean gastric wall mucus significantly increased after standard medication administration (fig. 3).

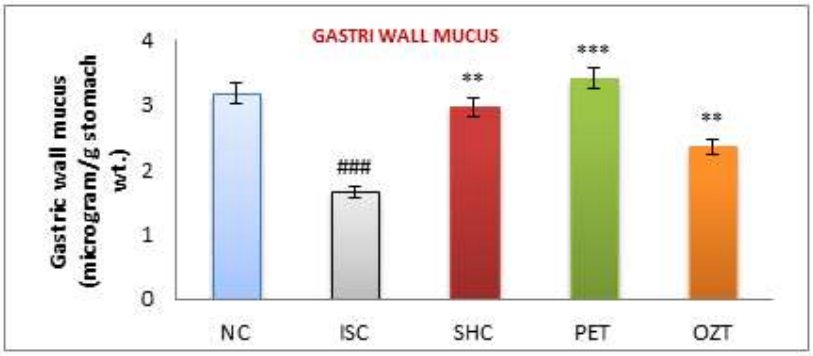


Figure 3: (A): Polygonum bistorta hydroalcoholic extract reduces the ulcer index in rats to treat stress-induced stomach ulcersmodel; (B): In immobilization stress-induced PUD rats, Polygonum bistorta extract improves gastric pathomorphology/ulcersModel Note: PET: Polygonum bistorta Extract Treated; SHC: Self-Healing Control; ISC: Immobilization Stress Control;OZT: Treated with Omeprazole. significantly different at ###p0.05 from NC rats.

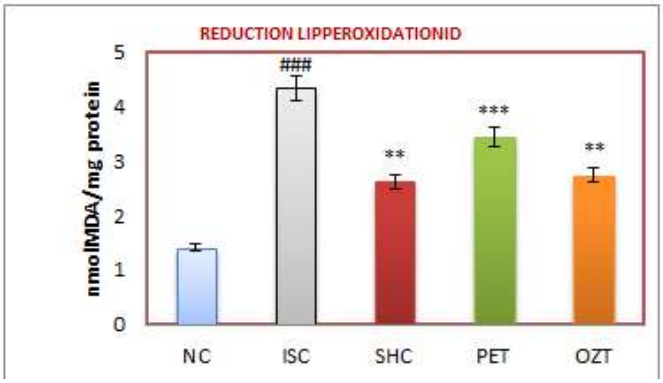


Figure 4: In immobilization, Polygonum bistorta extract significantly reduced lipid peroxidation events or MDA formation.Model of stomach ulcers caused by stress Note: Mean±SEM (n=5) is used to express the values (nmol MDA/mg protein). ISC PET: Polygonum bistorta Extract Treated; OZT, Omeprazole; SHC: Self-Healing Control; immobilization Stress-Control Treated; at ###p<0.01, significantly distinct from NC the rats

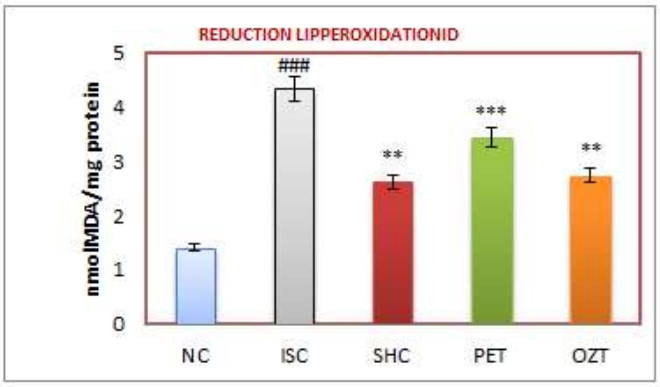
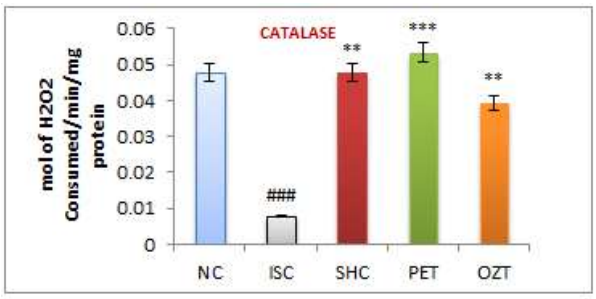
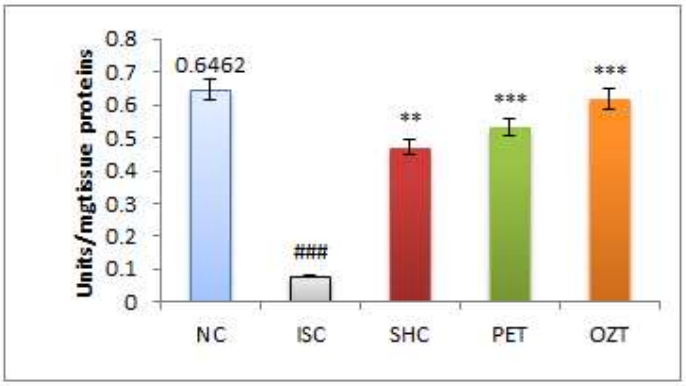


Figure 5: In the immobilization stress-induced gastric ulcer model, the administration of Polygonum bistorta extract clearly increased the gastric GSH content. Note: ISC stands for Immobilization Stress-Control, SHC for Self-Healing Control, PET for Polygonum bistorta Extract Treated, and OZT for Omeprazole Treated. Mean±SEM (n=5) is used to express the values (µg/mg protein); they differ significantly from NC rats at ###p<0.01.

Right now, interventional research estimated the activity of antioxidants with enzymes components alongwith the level of non-enzymatic antioxidants (GSH). Consequently, we noticed that the in comparison to the SSC group, CAT activity in stomach tissue dramatically dropped by 73.53%. Compared to the PUD rat model, the ISC (self-healing) group demonstrated a slight elevation in gastric CAT activity (1.53-fold) at the end of the study. However, when compared to the ISC group, the additives of Polygonum bistorta extract significantly increased the stomach CAT activity by 310.81%. Furthermore, PET treatment resulted in a 2.53-fold increase in CAT activity compared with the SHC group.. The most intriguing finding in this instance is that Polygonum bistorta extract increased CAT activity to the level observed in NC rats (nsp>0.05). Comparing ISC and SHC rats, the OZT treatment also reduced the gastric CAT activity by 181.08% and 73.33%, respectively; however, these results were not as noteworthy as previously notified.sin the PET group (fig. 6A)



(A)



(B)

Figure 6: Polygonum bistorta hydro-alcoholic extract improves the activity of enzymatic antioxidants to treat immobilization stress-induced gastric ulceration; (A) Effect of Polygonum bistorta extract on catalase (CAT) activity in an immobilization stress-induced peptic ulcer disease (PUD) rat model. CAT activity is expressed as nmol of H_2O_2 consumed/min/mg protein. Values are presented as mean ± SEM (n = 5).

(B) Effect of Polygonum bistorta extract on superoxide dismutase (SOD) activity in an immobilization stress-induced PUD rat model. SOD activity is expressed as units/mg tissue protein. Values are presented as mean ± SEM (n = 5).

Note: SSC: Immobilization Stress Control; NC: Normal Control; SHC: Self-Healing Control; PET: Polygonum bistorta Extract Treatment; OZT: Omeprazole Treatment.

Statistical significance:

###p < 0.001 vs NC;

p < 0.05, **p < 0.01, **p < 0.001 vs SHC;

#p < 0.05, ##p < 0.01, ###p < 0.001 vs ISC.

A 3-hour immobilization stress challenge significantly reduced gastric SOD activity by 52.72% compared to the normal control (NC) rats. In contrast, rats in the self-healing control (SHC) group exhibited a 36.69% increase in gastric SOD activity in the PUD model; however, this improvement remained considerably lower than that observed in the NC group.

Treatment with Polygonum bistorta extract (PET) markedly enhanced gastric SOD activity by 97.54% compared to the immobilization stress control (ISC) group. Additionally, statistical analysis showed that, compared to the SHC group, PET significantly enhanced the self-healing process, resulting in a 1.45-fold increase in SOD activity. Interestingly, no significant difference in SOD activity was observed between the NC and PET groups, suggesting potential restoration of antioxidant defense under stress-induced PUD conditions .Furthermore, omeprazole-treated (OZT) rats also showed an increase in gastric SOD activity; however, this effect was less pronounced compared to the PET-treated group (Fig. 6B).

Our H&E staining analyses revealed preserved gastric histoarchitecture in NC rats at the end of the study, accompanied by normal biochemical parameters. On the other hand, the ISC group's histopathological findings revealed severe damage to the stomach mucosa, the surface epithelium, and the sub-mucosal layer's swelling. Additionally, the micrographs of the untreated rats that were allowed to heal themselves showed a noticeably damaged stomach mucosa, surface epithelium, and oedematous spaces in the layer beneath the mucosa. However, the PET group's immobilization stress-induced PUD-related aberrant histoarchitecture was resolved by the Polygonum bistorta extract treatment.reduction of sub-mucosal oedema, epithelial cell losses, and damaged stomach mucosa when compared to micrographs from the ISC and SHC groups. On the other hand, the PET group's cure was also superior to the SHC group's, indicating Polygonum's therapeutic benefits. bistorta extract in treating stomach ulcers brought on by immobilization stress. Furthermore, histopathological analysis of the standard drug-treated group (OZT) showed that the gastric histoarchitecture returned to normal; however, OZT did not produce therapeutic effects comparable to the PET group (Fig. 7).

Researchers recognize the significant role of H. pylori in the development of peptic ulcer disease (PUD), as it occurs in the majority of intestinal and gastric ulcer cases. However, only 10%–20% of infected individuals develop PUD, indicating that additional factors contribute to disease onset. Epidemiological evidence shows that PUD also develops in 16%–30% of individuals without H. pylori infection or NSAID use, highlighting the involvement of alternative pathogenic mechanisms. Among these, psychophysiological stress plays a critical

role in ulcer development, and studies have established a significant correlation between stress and PUD. Despite this, researchers have not thoroughly investigated the influence of stress on PUD progression and its therapeutic modulation in currently available animal models. Therefore, the present study aimed to develop a novel post-treatment immobilization stress-induced PUD model and to evaluate the curative effects of Polygonum bistorta extract on various pathological parameters associated with PUD.

In a similar vein, we developed a novel post-treatment immobilization stress-induced PUD rat model for the first time. We induced PUD by immobilizing rats in vertical cylinders for three hours, followed by self-recovery for varying durations. We selected group III (sacrificed on day 14) as the experimental model, as this group exhibited visible gastric lesions even after 14 days of self-healing. Using this model, we evaluated the curative effect of Polygonum bistorta extract. Following a 3-hour immobilization stress challenge, we observed a significantly higher gastric ulcer index in ISC rats compared to NC rats at the end of the therapeutic intervention. his observation may be associated with increased gastric acid secretion induced by immobilization stress, which has been implicated in the pathogenesis of peptic ulcer disease (PUD; [11, 46, 47] Furthermore, stress-induced release of acetylcholine (ACh) activates the phospholipase C–protein kinase C (PKC) pathway, which ultimately stimulates gastric acid secretion and H^+/K^+-ATPase activity in parietal cell.[45, 46] In comparison to the SSC group, the self-healing observation group showed a lower ulcer index. In contrast to both the ISC and SHC groups, treatment with Polygonum bistorta extract considerably reduced the gastric ulcers caused by immobilization stress. The antioxidant and anti-inflammatory properties of Polygonum bistorta extract may be responsible for its healing effect on ulcer index. [47]

Mucus neck cells secrete mucus that protects the entire gastrointestinal epithelial lining. This mucus acts as a primary defensive barrier against ulcerogenic agents and pathogens, thereby preventing the development of ulcers, vascular damage, and mechanical injury. [12,50, 51] Glycoproteins, proteoglycans, and hyaluronic acid form the glycocalyx layer, which covers the endothelial surface of most organs. The glycocalyx layer protects gastric tissues from endogenous factors, such as gastric acid, pepsin, oxidized lipids, and bile salts, as well as from exogenous harmful agents, including alcohol, aspirin, and other nonsteroidal anti-inflammatory drugs NSAIDs. [50] [51] The present study's findings showed that ISC rats had significantly less gastric wall mucus than NC rats. Our findings are consistent with previous reports indicating reduced gastric wall mucus in various ulcer models, particularly indomethacin-induced PUD. [12,13] Furthermore, the PET group exhibited a significant increase in gastric mucus compared to the ISC and SHC groups, suggesting a potential role of Polygonum bistorta extract in promoting mucosal healing In SHC, the treatment produced a slight improvement in the amount of gastric wall mucus. Polygonum bistorta extract is rich in antioxidant secondary metabolites, such as ascorbic acid, gallic acid, and tannic acid, which may contribute to its gastroprotective effects. Additionally, its beneficial activity in the post-treatment immobilization stress–induced peptic ulcer disease (PUD) model may be associated with syringic acid .Previous reports have confirmed the positive effects of epicatechin and ellagic acid. [12, 13, 48] However, in the current PUD model, OZT also reduced the amount of mucus in the stomach wall, and these effects are consistent with previous reports. [12, 13]

MDA is a highly reactive and toxic carbonyl compound that is produced when conjugated dienes and lipid hydroperoxides undergo oxidative modification and degradation .[39, 40] Through nonenzymatic interactions with cellular macromolecules such as proteins, lipids, and DNA, carbonyl species contribute to cellular stress and toxicity in both in vitro and in vivo systems, leading to the formation of modified protein adducts and advanced glycation end products. [52 , 55] The concentration of malondialdehyde (MDA) in gastric mucosa has been extensively utilized as a biomarker of oxidative membrane damage in both experimental PUD models and human studies. [12, 31] Similarly, assessment of malondialdehyde (MDA) levels in gastric tissue revealed a significant increase in the ISC group, indicating that physiological stress enhances reactive oxygen species (ROS) production and induces membrane damage via lipid peroxidation. The administration of Polygonum bistorta extract significantly corrected the aforementioned aberrations, which may be due to its potent antioxidant potential, as demonstrated by the SHC group's somewhat improved MDA previous reports. [12,48]

Reactive oxygen species (ROS) are critically involved in the development of various pathological conditions, such as cancer,[65] neurological disorders,[65] renal diseases, [65] diabetes,[65,66] cardiovascular disorders (including atherosclerosis), [66] gastritis, and peptic ulcer disease.[67,68] Cells generate ROS in response to various stimuli and physiological demands, whereas the endogenous antioxidant defesnse system, comprising non-enzymatic (GSH) and enzymatic (CAT and SOD) components, neutralizes these reactive species. [66, 69] Conversely, oxidative imbalance, marked by increased oxidant levels and diminished antioxidant capacity, contributes to cellular and histological damage, lipid peroxidation, and the development of gastric ulcers [68,70]. In agreement with this, the ISC group exhibited decreased levels of nonenzymatic antioxidants, including glutathione (GSH) in gastric tissue.[68] This clearly demonstrates that the rats subjected to immobilization stress were unable to combat the increased generation of free radicals and the ensuing pathologies because their endogenous GSH levels were lower. In this study, immobilization-stress-induced oxidative stress caused

damage that even the self-healing strategy was unable to completely repair. In recent years, plant-based therapies have gained attention for the treatment of oxidative stress–induced peptic ulcer disease (PUD), primarily due to their potential to reduce the adverse effects associated with conventional synthetic drugs .[12,13,] We observed that Polygonum bistorta extract administration significantly elevated gastric glutathione (GSH) levels relative to the SHC group at the end of the intervention. Conversely, omeprazole treatment was associated with reduced GSH levels in gastric tissue in the immobilization stress–induced PUD rat model. These observations are in agreement with earlier studies. [12,13]

Redox homeostasis is maintained by enzymatic antioxidants, including catalase (CAT) and superoxide dismutase (SOD), which detoxify hydrogen peroxide (H_2O_2) and superoxide anion radicals, respectively, in conjunction with nonenzymatic antioxidants such as glutathione (GSH). [50,51] Thus, the gastric tissue of the interventional groups was examined for CAT and SOD activity. We found that the ISC group's CAT and SOD activities were markedly reduced in this attempt, which may be linked to increased ROS production and inflammatory cascades brought on by immobilization stress. Although the SHC group demonstrated slight, nonsignificant increases in CAT and SOD activities relative to the ISC group, treatment with Polygonum bistorta extract resulted in a marked elevation of these enzymes compared to SSC and SHC groups. This suggests a potential role of the extract's antioxidant metabolites in enhancing enzymatic antioxidant defense under stress-induced PUD conditions. These metabolites decreased oxidative stress and subsequently shielded the cellular membranes from damage caused by free radicals. The results of the present study are consistent with earlier reports demonstrating that treatment with diverse plant extracts significantly increases CAT and SOD activities in experimental models of peptic ulcer disease (PUD) .[12,13]

Histoarchitectural abnormalities in the stomach and other tissues are thought to be caused by physiological/oxidative stress in addition to biochemical parameters .[50,51] Similarly, extensive harm to the stomach lining, surface epithelium, and sub-mucosal layer oedema were revealed by histopathological analyses of gastric tissue from the ISC group. These anomalies may have been caused by acid secretion brought on by immobilization stress and oxidative stress (marked by changes in GSH and MDA levels as well as decreased CAT and SOD activities), which ultimately resulted in tissue destruction. Additionally, reduced gastric mucus made the stomach more vulnerable to attacks from free radicals and increased acid secretions, which resulted in abnormalities and destruction of the stomach histoarchitecture. Although self-healing was insufficient to fully cure these pathologies, Polygonum bistorta extract adjunct therapy reduced loss of epithelial cells and submucosal edema, thereby curing the aforementioned irregularities. On the other hand, the cure offered by Polygonum bistorta extract (PET) was also superior to that seen in the SHC group, indicating the extract's therapeutic benefits in treating gastric ulcers brought on by immobilization stress. Nevertheless, more clinical research is needed to support this formulation for the treatment of PUDs and validate the results of our current in vivo study. We created a rat model of PUD caused by post treatment immobilization stress in light of the value and need for animal models in the management and treatment of PUD. Polygonum bistorta extract reduced ulcer index, enhanced gastric mucus, and improved CAT, SOD, and GSH levels in the stress-induced PUD model, showing greater efficacy than omeprazole (Figure 8) and suggesting potential for clinical evaluation.

CONFLICTS OF INTERESTS

Authors declare that they have no conflict of interest.

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