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Pharmacological Evaluation of Stachydrine Supporting Multi-Pathway Intervention in Gastric Ulcer Disease

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Abstract

Gastric ulcer is a disease characterized from an imbalance between aggressive factors and mucosal defense system mechanisms, with oxidative stress, inflammation, and excessive gastric acid secretion playing central roles in tissue injury. The present study evaluated the gastroprotective potential of stachydrine using ethanol-induced and pyloric ligation ulcer models in mice. Gastric lesions were induced by absolute ethanol, while pyloric ligation was performed to assess gastric secretory parameters. Stachydrine (60 mg/kg) was administered orally, and its effect was compared with the standard drug Omeprazole. Ulcer index, gastric pH, gastric fluid volume, and oxidative stress markers, including glutathione and LPO, were evaluated. Stachydrine produced significant gastroprotection, reducing ulcer severity by up to 85% and significantly preserving mucosal integrity. In the ligation model, stachydrine elevated gastric pH and reduced gastric fluid volume, indicating moderate antisecretory activity. Ethanol exposure caused marked oxidative imbalance, characterized by depleted GSH and elevated LPO levels, whereas stachydrine restored antioxidant level and normalized LPO concentrations. These findings suggest that stachydrine mitigates gastric mucosal injury through antioxidant and anti-inflammatory mechanisms, along with partial suppression of gastric acid secretion. Stachydrine shown significant gastroprotective effects through multi-targeted and significant mechanisms. Given its protective ability to restore oxidative balance and improve gastric physiology, stachydrine may shown a significant

natural therapeutic candidate for gastric ulcer protection. Morover molecular and clinical investigations are required to validate its protective efficacy and signify its molecular impacts.

Introduction

Gastric ulcer is a common gastrointestinal disease supported by a localized erosion of the gastric mucosa that extends beyond the epithelial layer into the submucosa. It occurs when the balance between aggressive factors such as gastric acid and pepsin and protective mechanisms like mucus, bicarbonate, and adequate blood flow is disrupted (Sung et al., 2009). Clinically, patients often present with epigastric pain, nausea, bloating, and in severe cases, gastrointestinal bleeding or perforation (Laine et al., 2008). Several risk factors contribute to the development of gastric ulcers. Infection with Helicobacter pylori remains one of the leading causes worldwide, as it induces chronic inflammation and weakens mucosal defenses (Kusters et al., 2006). Prolonged use of nonsteroidal anti-inflammatory drugs (NSAIDs), including aspirin and ibuprofen, reduces prostaglandin synthesis, thereby impairing mucus production and mucosal protection (Wallace, 2008). Other contributing factors include smoking, excessive alcohol consumption, psychological stress, advanced age, and chronic systemic illnesses, all of which compromise gastric integrity and delay ulcer healing (Lanas & Chan, 2017). Globally, peptic ulcer disease affects millions of individuals, with a lifetime prevalence estimated at 5–10% (Sung et al., 2009). The burden remains significant in both developing and developed countries. In low- and middle-income regions, higher rates are largely attributed to persistent Helicobacter pylori infection and limited healthcare access (Hooi et al., 2017). In contrast, in developed countries, NSAID-associated ulcers are more prevalent due to widespread analgesic use (Lanas & Chan, 2017). Despite modern therapies, recurrence and drug-related adverse effects continue to pose clinical challenges. The role of Helicobacter pylori in ulcer pathogenesis is well established. This Gram-negative organism colonizes the gastric mucosa and survives acidic conditions by producing urease, which neutralizes gastric acid locally (Kusters et al., 2006). It releases virulence factors that promote epithelial injury, oxidative stress, and pro-inflammatory cytokine production. Chronic infection leads to sustained inflammation, disruption of mucosal barriers, and impaired regeneration, ultimately resulting in ulcer formation. Current treatment strategies focus on acid suppression, bacterial eradication, and mucosal protection. Proton pump inhibitors such as Omeprazole and Pantoprazole are widely prescribed to inhibit gastric acid secretion and promote healing (Scarpignato et al., 2016). Mucosal protective agents like sucralfate are also used, while combination antibiotic regimens are recommended for Helicobacter pylori eradication (Chey et al., 2017). Although effective, long-term therapy may be associated with side effects, antibiotic resistance, relapse, and altered gut microbiota, highlighting the need for safer and multi-targeted alternatives.

Natural bioactive compounds have gained increasing interest as potential therapeutic candidates. Stachydrine (Figure 1), an alkaloid isolated from Leonurus japonicus, has been extensively studied for its diverse pharmacological properties. Traditionally used in Asian medicine, stachydrine has demonstrated anti-inflammatory, anti-cancer, cardioprotective, and neuroprotective activities in various experimental models (Liu et al., 2010; Loh et al., 2019). Given that oxidative stress and inflammation are central to gastric ulcer pathogenesis, stachydrine pharmacological profile suggests potential therapeutic relevance. However, despite its wide range of reported biological activities, stachydrine has not yet been specifically investigated as a gastroprotective agent against gastric ulcer. Therefore, investigating its possible protective effects on gastric mucosa may provide novel and significant insights and contribute to the development of alternative therapeutic strategies for ulcer protection.

This preent study meets with the United Nations Sustainable Development Goal 3 (Good Health and Well-Being) by exploring safer and affordable therapeutic alternatives for gastric ulcer management.

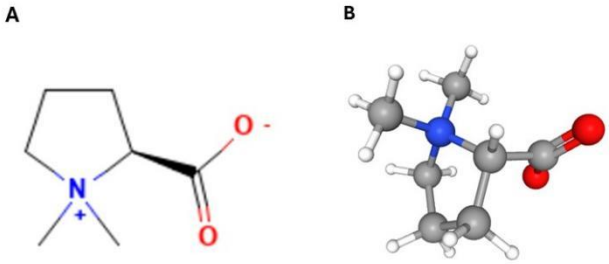

Figure 1: 2D and 3D structures of stachydrine

Material and Methods

Chemicals

All reagents and chemicals used in this study were obtained from reliable commercial suppliers. Stachydrine was procured from Sigma-Aldrich. Normal saline, absolute ethanol, and chloroform were also sourced from Sigma-Aldrich (St. Louis, MO, USA). Omeprazole was obtained from Barrett Hodgson Pakistan and Sanofi, respectively. All chemicals and reagents employed in the experiments were of analytical grade.

Animals

Healthy male and female mice weighing between 25-40 g were obtained from the National Institute of Health, Islamabad and used for this study. The animals were housed in standard laboratory cages under controlled environmental conditions, with the temperature maintained at 22 ± 2 °C. They were provided with a standard laboratory diet and allowed free access to water throughout the study period. All experimental procedures were conducted in accordance with the guidelines established by the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (1996), ensuring proper ethical care and handling of laboratory animals.

Ethanol-induced gastric ulcer

To induce gastric lesions, mice were fasted for 24 hours and then randomly divided into five groups (n = 5). Group 1 served as the saline control and received normal saline at 10 mL/kg body weight. Group 2 received ethanol (1 mL/100 g) and was considered the negative control. Groups 3 were pretreated orally with stachydrine at dose of 60 mg/kg, respectively, while Group 4 received omeprazole (20 mg/kg, p.o.) as the standard treatment. One hour after the respective treatments, all animals except those in the

control group were administered absolute ethanol (1 mL/100 g, oral) to induce gastric injury. After another hour, the mice were humanely euthanized by cervical dislocation. The stomachs were carefully removed, opened along the greater curvature, and examined for lesions. The ulcer index was determined by measuring the length of each lesion in millimeters. The affected areas were scored, measured, and documented for analysis (Li et al., 2021).

Pyloric ligation model

Pyloric ligation was carried out under light ether anesthesia, taking special care to avoid damage to the pylorus and surrounding blood vessels. After gently exteriorizing the stomach, the pyloric end was ligated, and the abdominal incision was carefully sutured. Following the procedure, the animals were deprived of food and water for a period of 4 hours. At the end of the experimental period, the animals were euthanized using an overdose of ether anesthesia followed by cervical dislocation. The stomachs were then removed and opened to collect the gastric contents. The collected material was centrifuged to separate solid debris, and the clear gastric juice was obtained for analysis. The volume and pH of the gastric secretions were subsequently measured and recorded (Azam et al., 2025).

Antioxidant assay

The stomach tissues from the mice were carefully homogenized and then centrifuged at 1500 rpm for 30 minutes. The resulting supernatant was collected and used for analysis. Levels of key antioxidant markers, including glutathione (GSH) and LPO, were measured within the supernatant (Sarkar et al., 2022).

Statistical Analysis

All data are presented as mean ± standard deviation (SD). Graphs and visual representations were prepared using GraphPad Prism (version 9.5.0 for macOS; GraphPad Software, San Diego, CA, USA), while statistical analyses were performed with SPSS (version 25 for macOS; IBM Corp., Armonk, NY, USA). Differences between groups were assessed using one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) post hoc test for multiple comparisons. Results were considered statistically significant when the p-value was less than 0.05.

Results

Effect on Ulcer Index

Stachydrine demonstrated notable anti-ulcer activity at dose of 60 mg/kg. At 60 mg/kg, it provided approximately 85% protection, corresponding to ulcer index scores of 2 ± 0.86 respectively. As a standard reference, omeprazole (20 mg/kg) exhibited a strong protective effect of 92%, reflected by an ulcer index of 1.4 ± 0.75, when compared to the ethanol-treated group (Table 1). Macroscopic examination of the gastric mucosa further confirmed these protective effects, as illustrated in (Figure 2).

Treatment (mg/kg)	Ulcer Index (UI)	% inhibition
Saline (10 mL/kg)	0 ± 0	-
Ethanol (1 mL/100 gm)	9.8 ± 1.4$^{###}$	0
Stachydrine (60 mg/kg) + ethanol (1 mL/100 gm)	2 ± 0.86***	85
Omeprazole (20 mg/kg) + ethanol (1 mL/100 gm)	1.4 ± 0.75***	92

TABLE 2. Protective effects of stachydrine and omeprazole against ethanol-induced gastric ulcer in mice.

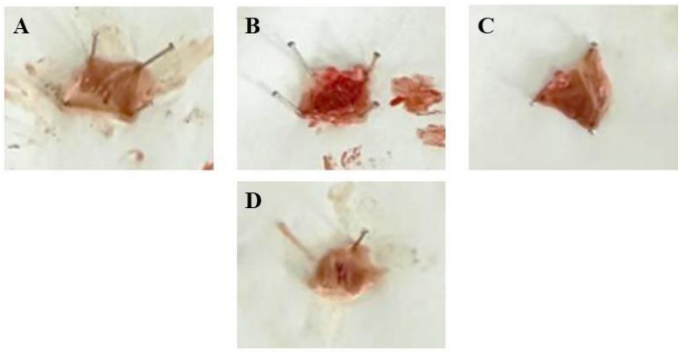

Figure 2: Gross appearance of gastric mucosa in mice. (A) Control group pretreated with saline (10 mL/kg) showing intact mucosa. (B) Ethanol-treated group (1 mL/100 g) exhibiting severe gastric lesions. (C) Group pretreated with stachydrine at 60 mg/kg, respectively, showing significant protection of the gastric lining. (D) Omeprazole-treated group (20 mg/kg) serving as the standard reference, demonstrating significant mucosal protection.

Stomach pH and gastric volume

In the pyloric ligation model, the saline-treated animals maintained near-physiological conditions, showing a gastric pH of 3.19 ± 0.13 and a gastric fluid volume of 1.58 mL. In contrast, ethanol administration markedly increased gastric acidity, reducing the pH to 2.38 ± 0.19 while elevating gastric fluid volume to 2.73 mL. Treatment with Omeprazole (20 mg/kg) significantly increased gastric pH to 7.41 ± 0.14 and lowered fluid volume to 1.31 mL and stachydrine at 60 mg/kg improved gastric conditions, raising the pH to 6.58 ± 0.36 and reducing gastric volume to 1.37 mL (Table 2).

Table 2: Effects of stachydrine on gastric pH and gastric fluid volume.

Groups	PH	Gastric Content volume/ml
Saline (10 mL/kg)	3.19 ± 0.13	1.58 ± 0.12
Ethanol (1 mL/100 gm)	2.38 ± 0.19	2.73 ± 0.28
Stachydrine (60 mg/kg) + ethanol (1 mL/100 gm)	6.58 ± 0.36	1.37 ± 0.07
Omeprazole (20 mg/kg) + ethanol (1 mL/100 gm)	7.41 ± 0.14	1.31 ± 0.08

Effect on oxidative stress

In gastric tissues from the ethanol-induced ulcer group, glutathione (GSH) levels were markedly depleted, while LPO levels were elevated, indicating oxidative imbalance. Treatment with stachydrine (60 mg/kg) and Omeprazole (20 mg/kg) restored redox balance by lowering LPO levels and simultaneously enhancing GSH content (Figure 3).

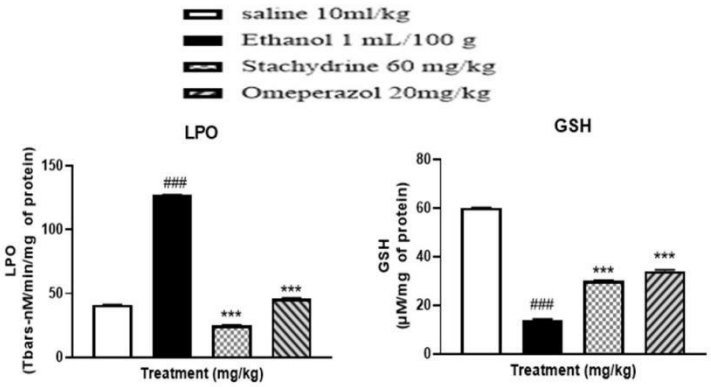

Figure 7: Significant Effects of stachydrine and omeprazole against GSH and LPO in ethanol induced gastric ulcer tissues using antioxidant assay.

Discussion

Gastric ulcer is the most prominent disease characterized by inflammation, excessive acid secretion, and compromised mucosal defense collectively contribute to tissue demage. The present study demonstrates that stachydrine exerts significant gastroprotective effects in both ethanol-induced and pyloric ligation models in mice. The findings suggest that stachydrine not only reduces visible gastric lesions but also improves gastric secretory parameters and restores oxidative balance, indicating a multi-targeted protective mechanism. The ethanol-induced ulcer model is widely used to evaluate cytoprotective agents because ethanol rapidly penetrates the gastric mucosa, causing necrotic lesions, vascular damage, and intense oxidative stress (Sung et al., 2009). Ethanol disrupts the mucus-bicarbonate barrier, increases lipid peroxidation, and promotes inflammatory mediator release, ultimately leading to hemorrhagic lesions. In the present study, ethanol administration produced a marked increase in ulcer index, confirming severe mucosal injury. Pretreatment with stachydrine significantly reduced lesion severity achieving up to 85% protection at 60 mg/kg. This protective efficacy, although slightly lower than that of omeprazole, was substantial and biologically meaningful. Proton pump inhibitors such as Omeprazole act primarily by irreversibly inhibiting the H^+/K^+-ATPase enzyme in parietal cells, thereby suppressing gastric acid secretion (Scarpignato et al., 2016). While highly effective, long-term PPI use has been associated with adverse outcomes, including altered gut microbiota and increased infection risk. In contrast, natural compounds like leonurine may offer broader protective effects through antioxidant and anti-inflammatory pathways in addition to moderate acid suppression. The pyloric ligation model further clarified stachydrine antisecretory potential. Ethanol exposure significantly reduced gastric pH and increased gastric fluid volume, reflecting enhanced acidity and secretory activity. Stachydrine treatment elevated gastric pH and reduced gastric content volume, suggesting partial inhibition of gastric acid secretion. Although its effect was slightly less pronounced than that of omeprazole, stachydrine demonstrated substantial modulation of gastric physiology. These findings indicate that stachydrine anti-ulcer activity may involve both cytoprotective and antisecretory mechanisms. Oxidative stress is a central mediator in gastric ulcer pathogenesis. Ethanol-induced injury is closely linked to excessive production of reactive oxygen species (ROS), leading to depletion of endogenous antioxidants such as glutathione (GSH) (Kusters et al., 2006). Reduced GSH levels impair cellular defense mechanisms and enhance lipid peroxidation, thereby worsening mucosal damage. In this study, ethanol markedly depleted GSH and elevated LPO levels, reflecting oxidative and nitrosative stress. Stachydrine treatment

significantly restored GSH levels while reducing LPO concentrations, indicating improved redox balance. These findings align with previous reports describing stachydrine antioxidant and free radical-scavenging properties (Liu et al., 2010). Nitric oxide plays a dual role in gastric physiology. At physiological levels, LPO maintains mucosal blood flow and promotes mucus secretion. However, excessive LPO production particularly via inducible nitric oxide synthase (iNOS)—contributes to inflammatory damage and oxidative stress (Wallace, 2008). The reduction of elevated NO levels by stachydrine in this study suggests modulation of inflammatory signaling pathways, possibly through inhibition of pro-inflammatory mediators. Previous research has shown that leonurine suppresses NF-κB activation and reduces inflammatory cytokine production, supporting this interpretation (Loh et al., 2019). The gastroprotective effects observed may therefore result from a combination of mechanisms attenuation of oxidative stress through restoration of GSH, reduction of excessive LPO and inflammatory signaling, and partial suppression of gastric acid secretion. Such multi-targeted activity is particularly valuable in ulcer management, where pathogenesis involves overlapping pathways including acid aggression, impaired mucosal defense, and inflammation (Lanas & Chan, 2017). Importantly, although stachydrine has been extensively studied for cardioprotective, neuroprotective, and anti-inflammatory activities, its role in gastric ulcer has not been well characterized. The present findings provide initial experimental evidence supporting its gastroprotective potential. Further experimentation are neccessory to elucidate its precise molecular mechanisms, including evaluation of prostaglandin synthesis, mucus production and inflammatory levels.

Conclusion

Stachydrine showed notable gastroprotective effects by decreasing ulcer severity, enhancing gastric pH, and restoring antioxidant balance. Its protective activity seems to be mediated through antioxidant, anti-inflammatory, and mild antisecretory actions. While slightly less potent than Omeprazole, stachydrine still provided significant mucosal protection. These results highlight stachydrine as a promising natural compound for further exploration in the management of gastric ulcers.

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