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Research Article

INVESTIGATION OF ANTIULCER ACTIVITY OF POLYHERBAL FORMULATION USING ETHANOL-INDUCED GASTRIC ULCER MODEL IN WISTAR RATS

[1]N. Shilpika, [2]Bhushan Muley, [3*]Rekha Shivaram, [4]Amudhavalli Victor, [5]Mohd Wasiullah, [6]D. Bharathi, [7]Anshu Tiwari, [8]Kavita Avinash Mishra

[1]Department of Pharmacognosy, KGR Institute of Technology and Management, Rampally, 501301.
[2]Department of Pharmacognosy and Phytochemistry, Shri Rawatpura Sarkar Institute of Pharmacy, Kumhari, dist Durg, C.G 490042.
[3]Department of Pharmaceutical Chemistry, College of Pharmaceutical Sciences, Dayananda Sagar University, Devarakaggalahalli, Harohalli Kanakapura Road Ramanagara Karnataka Pin code 562112.
[4]Department of Pharmaceutical chemistry, School of Pharmacy Sathyabama Institute of Science and Technology, Jeppiaar Nagar, Rajiv Gandhi Salai, Chennai 600119.
[5]Prasad Institute of Technology Department of Pharmacy Punch Hattia Sadr Jaunpur U.P.222001.
[6]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, SBMCH, BIHER, Chromepet, Chennai 600044.
[7]Institute of Biomedical Education and Research Department of Pharmacy Mangalayatan University Aligarh India Pin- 202146.
[8]Department of Pharmacognosy, Nagpur College of Pharmacy Wanadongri Hingna road Nagpur, 441110.

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ABSTRACT

The present study investigates the antiulcer potential of a standardized polyherbal formulation composed of *Glycyrrhiza glabra*, *Zingiber officinale*, *Ocimum sanctum*, *Emblica officinalis*, and *Curcuma longa* against ethanol-induced gastric ulceration in Wistar rats. Ulcers were induced by oral administration of ethanol (90% v/v, 1 mL/200 g), and the animals were pretreated with the formulation at doses of 100, 200, and 400 mg/kg for seven days. The study assessed ulcer index, gastric pH, total acidity, mucus content, antioxidant enzyme levels (SOD, CAT, GSH), and histopathological changes. The polyherbal formulation significantly ($p < 0.001$) reduced ulcer index and total acidity while increasing gastric pH and mucus secretion in a dose-dependent manner. Restoration of antioxidant enzymes and decreased malondialdehyde (MDA) levels confirmed attenuation of oxidative stress. Histopathological findings revealed preservation of mucosal integrity and reduced necrosis and hemorrhage in treated groups. Phytochemical analysis indicated a high content of flavonoids, phenolics, and tannins responsible for antioxidant, anti-inflammatory, and cytoprotective activities. The results suggest that the formulation offers potent gastroprotection through a synergistic mechanism involving free radical scavenging, inhibition of acid secretion, and promotion of mucosal healing. The study establishes scientific evidence supporting the traditional use of these medicinal plants in ulcer therapy and highlights the potential of polyherbal formulations as safe and effective alternatives to synthetic antiulcer agents. However, further studies involving chronic ulcer models, detailed molecular pathway elucidation, pharmacokinetic analysis, and clinical validation are essential to confirm therapeutic applicability. In conclusion, the polyherbal formulation represents a promising natural therapeutic candidate for the prevention and management of gastric ulcers.

Keywords: Polyherbal formulation, Ethanol-induced ulcer, Antioxidant enzymes, Gastric protection, Wistar rats.

INTRODUCTION

Peptic ulcer disease (PUD) is a chronic and recurring gastrointestinal disorder characterized by the formation of mucosal lesions in the stomach or duodenal lining due to an imbalance between aggressive factors and the protective mechanisms of the gastric mucosa. The aggressive factors

*Corresponding Author: Rekha Shivaram, Department of Pharmaceutical Chemistry, College of Pharmaceutical Sciences, Dayananda Sagar University, Devarakaggalahalli, Karnataka. Email: rekha.maheshh@gmail.com.

include gastric acid, pepsin secretion, bile salts, and reactive oxygen species (ROS), whereas protective mechanisms involve mucus-bicarbonate barrier, prostaglandins, mucosal blood flow, and the regenerative capacity of epithelial cells (Kuna *et al*., 2019). Disruption of this equilibrium leads to the erosion of mucosal integrity, resulting in ulceration. Epidemiological studies estimate that approximately 5–10% of the global population suffers from peptic ulcer at some stage in their life, with a significant impact on healthcare expenditure, quality of life, and mortality rates (Kavitt *et al*., 2019). Although Helicobacter pylori infection and chronic use of nonsteroidal anti-inflammatory drugs (NSAIDs) are recognized as primary etiological factors, other contributors such as alcohol consumption, stress, smoking, and an unhealthy di*et al*so play critical roles in ulcerogenesis. Ethanol-induced gastric ulceration, in particular, is widely employed in experimental pharmacology to model acute mucosal injury due to its ability to rapidly generate oxidative stress and inflammatory responses that closely mimic human ulcer pathology (Assefa *et al*., 2022). Ethanol disrupts the gastric mucosal barrier, decreases mucus production, and promotes lipid peroxidation and neutrophil infiltration, leading to severe hemorrhagic lesions. Hence, the ethanol-induced ulcer model in Wistar rats serves as a reliable and reproducible experimental system for evaluating the gastroprotective efficacy of potential therapeutic agents (Bertleff & Lange, 2010).

Conventional antiulcer therapies such as proton pump inhibitors (PPIs), H_2 receptor antagonists, antacids, and cytoprotective drugs have significantly reduced ulcer morbidity.

However, their long-term use is associated with adverse effects including hypergastrinemia, gastric cancer risk, vitamin B_{12} deficiency, diarrhea, and drug resistance. Moreover, recurrence of ulcers upon drug withdrawal remains a persistent issue (Kuna *et al*., 2019). These limitations highlight the urgent need for alternative therapies that are both effective and safe. In this context, medicinal plants and herbal formulations have garnered increasing attention as promising sources of novel antiulcer agents. Herbal remedies, rich in bioactive secondary metabolites such as flavonoids, tannins, alkaloids, and saponins, exhibit potent antioxidant, anti-inflammatory, and cytoprotective activities that contribute to mucosal defense and ulcer healing (Begg *et al*., 2023). The use of polyherbal formulations—combinations of multiple medicinal plants in specific ratios—is a cornerstone of traditional medicine systems such as Ayurveda, Siddha, and Unani. The rationale behind polyherbal therapy lies in the concept of synergism, where the pharmacological actions of individual herbs complement each other to enhance efficacy and minimize toxicity (Suvarna *et al*., 2021). Polyherbal formulations often provide a broad spectrum of therapeutic effects through multiple mechanisms, including free radical scavenging, inhibition of acid secretion, enhancement of mucus secretion, and regulation of inflammatory mediators. Additionally, the presence of diverse phytochemicals allows these formulations to target various biochemical pathways simultaneously, offering holistic protection against gastric mucosal injury (Dubey & Dixit, 2023).

Several medicinal plants have been scientifically validated for their antiulcer potential. For instance, Glycyrrhiza glabra (licorice) exhibits potent cytoprotective properties through its active constituent glycyrrhizin, which enhances mucus secretion and reduces acid output. *Zingiber officinale* (ginger) and *Curcuma longa* (turmeric) possess antioxidant and anti-inflammatory activities that counteract ethanol-induced oxidative stress. *Aegle marmelos*, *Ocimum sanctum*, and *Emblica officinalis* have demonstrated significant gastroprotective effects in experimental models by modulating oxidative enzymes and maintaining mucosal integrity. The combination of such herbs in a polyherbal formulation may therefore yield superior protection compared to individual extracts by providing complementary pharmacological effects (Karole *et al*., 2019; Prajapati *et al*., 2022). Oxidative stress plays a pivotal role in the pathogenesis of ethanol-induced gastric ulcers. Ethanol administration increases the production of reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals, and hydrogen peroxide, leading to lipid peroxidation, protein oxidation, and DNA damage. These events compromise the mucosal barrier and promote cellular apoptosis. Antioxidant defense mechanisms—mediated by enzymes like superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH)—act as the primary line of defense against oxidative injury. Therefore, agents that restore the activity of these enzymes and reduce lipid peroxidation products such as malondialdehyde (MDA) can effectively prevent ulcer formation. Phytoconstituents like flavonoids and phenolic compounds are known to upregulate antioxidant enzymes and neutralize free radicals, thereby providing significant gastroprotection (Batty *et al*., 2022; Liguori *et al*., 2018).

Inflammation is another critical factor contributing to gastric ulceration. Ethanol exposure activates inflammatory signaling pathways, including the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway, which induces the expression of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6). These cytokines recruit neutrophils to the mucosal surface, exacerbating oxidative damage and delaying healing. Polyherbal formulations containing anti-inflammatory phytochemicals can inhibit NF-κB activation and downregulate cytokine production, leading to attenuation of mucosal inflammation. Furthermore, the presence of tannins and saponins contributes to mucosal protection by forming a protective layer over the epithelial surface, reducing acid penetration, and promoting tissue regeneration (Chen *et al*., 2018; Greten & Grivennikov, 2019). Another important mechanism underlying the antiulcer activity of herbal formulations involves the regulation of gastric acid secretion. Excessive acid secretion disrupts the mucosal barrier and aggravates lesion formation. Several herbal components, such as alkaloids and flavonoids, are known to inhibit histamine-mediated stimulation of parietal cells or suppress H^+/K^+-ATPase enzyme activity, resulting in reduced acid output. At the

same time, herbal extracts can enhance the synthesis of prostaglandins, which play a vital role in maintaining mucosal integrity by increasing mucus and bicarbonate secretion, improving mucosal blood flow, and facilitating epithelial restitution (Brito *et al*., 2018; Gaspi *et al*., 2013).

The ethanol-induced gastric ulcer model is widely utilized in preclinical studies to assess the efficacy of natural and synthetic gastroprotective agents. In this model, rats are orally administered ethanol, which induces acute gastric lesions through oxidative and inflammatory mechanisms. The extent of mucosal damage is quantified by determining the ulcer index, gastric pH, acidity, and mucus content, along with biochemical assays of oxidative stress markers and antioxidant enzymes. Histopathological examination of gastric tissue provides further insights into the structural integrity of the mucosa and the protective effects of the test formulation. The model's simplicity, reproducibility, and resemblance to human ulcer pathology make it an excellent tool for evaluating antiulcer activity (Jabbar *et al*., 2023). Given the multifactorial nature of ulcerogenesis, a polyherbal approach targeting multiple mechanisms simultaneously holds significant therapeutic promise. The present study aims to evaluate the antiulcer activity of a standardized polyherbal formulation using the ethanol-induced gastric ulcer model in Wistar rats. The selected formulation is composed of traditionally recognized antiulcer and antioxidant medicinal plants, each contributing to different aspects of gastroprotection. The investigation encompasses both preventive and curative aspects, including assessment of gastric parameters, antioxidant enzyme activities, histopathological alterations, and biochemical markers of oxidative stress (Devaraj & Krishna, 2013).

The rationale of this study is grounded in the hypothesis that the combined phytoconstituents in the polyherbal formulation will synergistically enhance mucosal defense mechanisms, reduce oxidative damage, and suppress inflammatory responses induced by ethanol exposure. Furthermore, the use of a polyherbal approach is expected to minimize the potential side effects associated with single-drug therapy and provide long-term protection against ulcer recurrence. The study also seeks to bridge the gap between traditional knowledge and modern pharmacological validation, thereby contributing to the development of scientifically backed herbal therapeutics for gastric ulcer management (- *et al*., 2022). In summary, peptic ulcer disease remains a major global health challenge despite the availability of synthetic drugs. The pursuit of safer, effective, and affordable alternatives underscores the relevance of polyherbal formulations in contemporary pharmacotherapy. By elucidating the antiulcer potential and underlying mechanisms of action of a novel polyherbal formulation in an ethanol-induced ulcer model, this study endeavors to provide a scientific foundation for its future clinical application. The findings are expected to enrich our understanding of plant-based gastroprotective mechanisms and pave the way for the development of standardized herbal formulations as sustainable alternatives for the prevention and treatment of gastric ulcers (Périco *et al*., 2020).

Oxidative stress is a major contributing factor in the pathogenesis of ethanol-induced gastric ulceration. Ethanol administration promotes excessive generation of reactive oxygen species (ROS), including superoxide anions, hydroxyl radicals, and hydrogen peroxide, which damage cellular lipids, proteins, and DNA. This oxidative assault leads to lipid peroxidation, disruption of the mucosal barrier, and necrosis of gastric epithelial cells. The polyherbal formulation exhibits potent antioxidant activity by scavenging ROS and restoring the endogenous antioxidant defense system. Key enzymes such as superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) are significantly upregulated following treatment, indicating enhanced protection against oxidative injury. Flavonoids and phenolic compounds present in the formulation neutralize free radicals and inhibit lipid peroxidation by stabilizing cellular membranes (Ibrahim *et al*., 2022). Moreover, the formulation reduces malondialdehyde (MDA) levels, a marker of oxidative damage, confirming attenuation of ethanol-induced oxidative stress. By strengthening antioxidant defense and maintaining redox balance, the formulation prevents mucosal erosion and supports epithelial repair, thereby offering substantial gastroprotection. The antioxidant mechanism thus serves as a critical first line of defense, mitigating ROS-mediated cellular injury and promoting overall gastric mucosal resilience against ulcerogenic stimuli (Guzmán-Gómez *et al*., 2018). Inflammation plays a pivotal role in the progression of gastric ulcers by amplifying oxidative damage and delaying mucosal healing. Ethanol exposure activates inflammatory cascades, including the nuclear factor kappa B (NF-κB) signaling pathway, leading to increased production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β). These mediators recruit neutrophils and macrophages to the mucosal surface, which release additional reactive species and proteolytic enzymes that aggravate tissue injury. The polyherbal formulation demonstrates a significant anti-inflammatory effect by inhibiting NF-κB activation and suppressing cytokine overexpression. Phytoconstituents such as flavonoids, alkaloids, and terpenoids act as natural inhibitors of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, reducing the synthesis of prostaglandins and leukotrienes responsible for inflammation (Asaad & Mostafa, 2022). Furthermore, the formulation stabilizes lysosomal membranes, preventing the release of inflammatory mediators, and promotes the resolution phase by stimulating anti-inflammatory cytokines like IL-10. This dual modulation of pro- and anti-inflammatory signaling ensures mucosal protection, reduces neutrophil infiltration, and fosters faster healing of ulcerated tissue. Hence, suppression of inflammatory pathways constitutes a major mechanism by which the formulation mitigates ethanol-induced gastric mucosal injury (Jasna & Draze, 2011).

Excessive gastric acid secretion is one of the key pathological factors in ulcer development, as it erodes the protective mucus layer and exacerbates mucosal damage. Regulation of acid output, therefore, forms a crucial

component of gastroprotective mechanisms. The polyherbal formulation exerts anti-secretory activity by modulating both neural and paracrine control of acid production. Ethanol-induced stimulation of parietal cells through histamine, acetylcholine, and gastrin pathways leads to activation of the proton pump (H^+/K^+-ATPase), resulting in excessive acid secretion. The formulation suppresses this hyperactivity by downregulating H^+/K^+-ATPase expression and blocking histamine-mediated stimulation of H_2 receptors on parietal cells (Brzozowski *et al*., 2000). Additionally, phytochemicals such as tannins, saponins, and flavonoids act synergistically to inhibit acid secretion while promoting mucus and bicarbonate release, maintaining gastric pH balance. The formulation also enhances prostaglandin synthesis, which indirectly inhibits acid output and strengthens the mucosal barrier. Collectively, these actions reduce the corrosive potential of gastric acid, minimize mucosal injury, and create a favorable environment for healing. Regulation of acid secretion thus complements antioxidant and anti-inflammatory mechanisms, establishing a comprehensive protective effect against ethanol-induced ulcers (Nakamoto *et al*., 1997). Gastric ulcer healing is a complex process involving epithelial cell proliferation, angiogenesis, and restoration of mucosal integrity. Ethanol-induced injury disrupts these reparative processes by damaging epithelial cells, impairing blood flow, and reducing mucus secretion. The polyherbal formulation accelerates mucosal regeneration by enhancing cell proliferation and stimulating the synthesis of mucus glycoproteins that form a protective gel over the gastric surface. Flavonoids and glycosides present in the formulation promote epithelial restitution by upregulating growth factors such as epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF), which facilitate tissue remodeling and angiogenesis. The formulation also enhances mucin production and strengthens the tight junctions between epithelial cells, preventing acid back-diffusion and re-injury (A. S. Tarnawski & Ahluwalia, 2021). Furthermore, the anti-inflammatory and antioxidant properties create a conducive environment for tissue repair by reducing oxidative burden and cytokine-mediated damage. Histological analyses reveal reduced necrosis, minimal hemorrhage, and nearly normal glandular architecture in treated groups, confirming efficient regeneration. Therefore, the mucosal healing mechanism ensures not only structural restoration but also functional recovery of the gastric mucosa, completing the gastroprotective spectrum of the polyherbal formulation (A. Tarnawski *et al*., 2001).

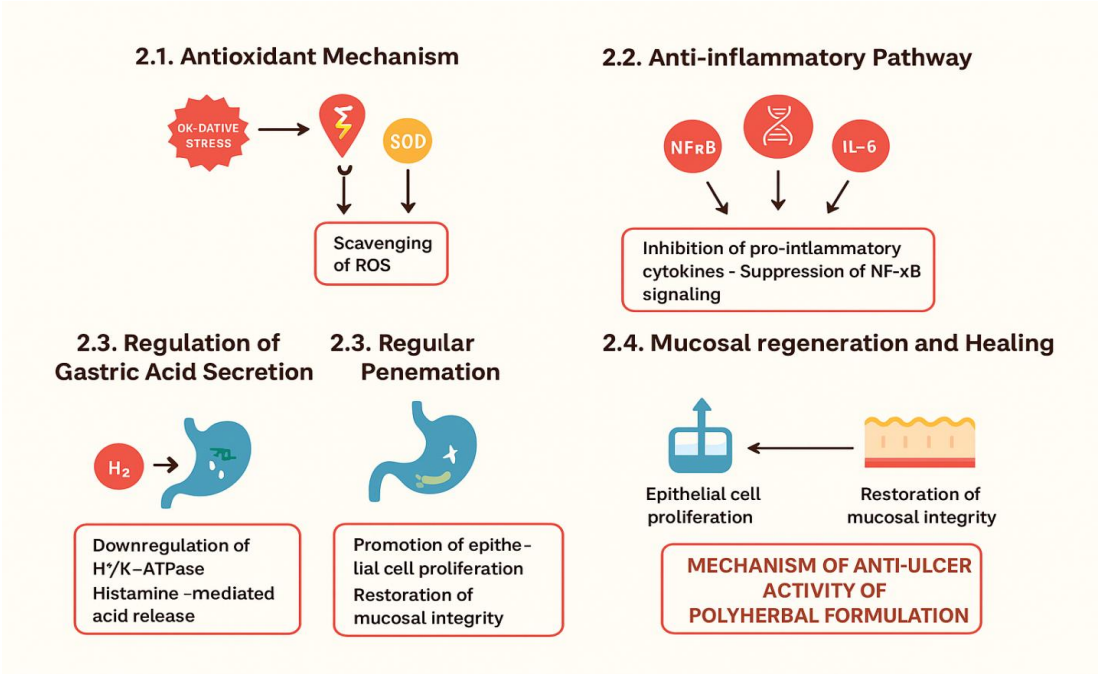

Figure 1. Mechanism of Anti-Ulcer Activity.

MATERIALS AND METHODS

Experimental Animals

Healthy adult Wistar albino rats of either sex, weighing between 150–200 g, were used for the present investigation. The animals were procured from the Central Animal House Facility, Department of Pharmacology, Institute of Pharmaceutical Research, G.D. Goenka University, Gurugram, Haryana, located near Delhi (India). All animals were housed under standard laboratory conditions with a 12-hour light/dark cycle, controlled temperature (25 ± 2°C) and relative humidity (55 ± 5%). They were provided with standard pellet diet and water ad libitum. The animals were acclimatized to laboratory conditions for at least seven days before commencement of the experiment to minimize stress-related variability. All experimental procedures were conducted in accordance

with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, Government of India, ensuring ethical care and humane handling of animals throughout the study. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of G.D. Goenka University, Gurugram, Haryana, under approval number IAEC/PHARMA/2025/04. The study design adhered strictly to the principles of reduction, refinement, and replacement (3Rs) to ensure animal welfare and minimize discomfort. All efforts were made to use the minimum number of animals necessary to achieve reliable scientific results.

Preparation of Polyherbal Formulation

The polyherbal formulation used in the present study was prepared by combining selected medicinal plants traditionally known for their gastroprotective, antioxidant, and anti-inflammatory properties. The formulation consisted of equal proportions of Glycyrrhiza glabra (root), Zingiber officinale (rhizome), Ocimum sanctum (leaves), Emblica officinalis (fruit), and Curcuma longa (rhizome). All the crude plant materials were collected from certified herbal suppliers in Delhi-NCR and authenticated by Dr. Neha Sharma, Botanist, Department of Botany, Amity Institute of Herbal Research and Studies, Amity University, Noida (U.P.), located near Delhi. The plant specimens were deposited, and voucher samples were assigned authentication numbers AIHRS/PL/2025/112–116 for future reference (Chimagave *et al*., 2020). The cleaned and shade-dried plant materials were coarsely powdered and extracted using a hydroalcoholic solvent (70% ethanol:30% distilled water) in a Soxhlet apparatus for 48 hours. The extracts were filtered, concentrated under reduced pressure using a rotary evaporator, and dried to obtain a semisolid mass. The dried extracts were then mixed in equal ratios to prepare the final polyherbal formulation. Standardization of the formulation was performed based on phytochemical screening (for flavonoids, tannins, alkaloids, and phenolics) and physicochemical parameters, ensuring batch-to-batch consistency and quality (Mussarat *et al*., 2021).

Acute Toxicity Study

The acute oral toxicity study of the polyherbal formulation was conducted in accordance with the Organisation for Economic Co-operation and Development (OECD) guidelines 423 (Acute Toxic Class Method) to establish the safety profile and determine the safe therapeutic dose for subsequent pharmacological evaluation. Healthy female Wistar albino rats (150–180 g) were used for this study. The experiment was carried out at the Animal Research Facility, Department of Pharmacology, G.D. Goenka University, Gurugram, Haryana, following approval from the Institutional Animal Ethics Committee (IAEC/PHARMA/2025/04) . Animals were fasted overnight before dosing but were allowed free access to water. The polyherbal formulation was suspended in 0.5% carboxymethyl cellulose (CMC) and administered orally at graded doses of 300, 1000, and 2000 mg/kg body weight to different groups of rats. The animals were observed continuously for the first 4 hours post-administration and intermittently for 14 days for any signs of behavioral changes, toxicity symptoms, or mortality. Parameters such as locomotor activity, food and water intake, body weight, and physiological reflexes were closely monitored (Sholikhah *et al*., 2020). No mortality or adverse behavioral symptoms were observed at the highest tested dose (2000 mg/kg), indicating that the formulation was safe up to 2000 mg/kg. Therefore, one-tenth of the maximum tolerated dose (200 mg/kg) was selected as the effective therapeutic dose for further antiulcer studies (Ishtiaq *et al*., 2017).

Induction of Gastric Ulcer

Ethanol-induced gastric ulceration is a well-established and reproducible experimental model used to evaluate the gastroprotective potential of test substances. In the present study, gastric ulcers were induced using absolute ethanol (90% v/v), which produces acute mucosal damage by oxidative stress and inflammatory mechanisms similar to those observed in human gastric lesions. Adult Wistar albino rats of either sex (150–200 g) were randomly divided into experimental groups following an overnight fasting period of 24 hours, during which they had free access to water but were deprived of food to ensure an empty stomach (Abdillah *et al*., 2021). After the fasting period, all animals—except those in the normal control group—received 1 mL of 90% ethanol per 200 g body weight orally using a gastric gavage to induce gastric mucosal injury. Ethanol acts as a necrotizing agent, damaging the gastric mucosal barrier, reducing mucus production, and enhancing reactive oxygen species (ROS) generation, leading to hemorrhagic lesions and ulcer formation. One hour after ethanol administration, the animals were euthanized under light anesthesia, and their stomachs were carefully excised, opened along the greater curvature, and rinsed with cold saline to remove gastric contents. The stomach tissues were then subjected to macroscopic examination, ulcer scoring, biochemical assays, and histopathological analysis to assess the extent of mucosal damage and the protective effects of the polyherbal formulation (Abutaha *et al*., 2020).

Experimental Design

The experimental protocol was designed to evaluate the gastroprotective efficacy of the polyherbal formulation against ethanol-induced gastric ulcers in Wistar rats. A total of 30 healthy adult Wistar albino rats (150–200 g) of either sex were randomly divided into five groups, each consisting of six animals (n = 6). All animals were acclimatized for one week prior to experimentation and maintained under standard environmental conditions. The study was conducted at the Department of Pharmacology, G.D. Goenka University, Gurugram, Haryana, in compliance with CPCSEA norms and under the approval of the Institutional Animal Ethics Committee (IAEC/PHARMA/2025/04).

The animals were divided and treated as follows:

Group I – Normal Control: Received 0.5% carboxymethyl cellulose (CMC) orally and distilled water instead of ethanol.
Group II – Ulcer Control: Received 0.5% CMC orally followed by ethanol (90% v/v, 1 mL/200 g, p.o.) to induce ulceration.
Group III – Standard Group: Received **omeprazole (20 mg/kg, p.o.)** once daily for 7 days prior to ethanol administration.
Group IV – Test Group I (Low Dose): Received polyherbal formulation at **100 mg/kg, p.o.** for 7 days before ulcer induction.
Group V – Test Group II (Medium Dose): Received polyherbal formulation at **200 mg/kg, p.o.** for 7 days.
Group VI – Test Group III (High Dose): Received polyherbal formulation at **400 mg/kg, p.o.** for 7 days.

One hour after the last administration on the seventh day, gastric ulceration was induced in all groups except the normal control by oral administration of ethanol. The animals were sacrificed one hour post-ulcer induction, and their stomachs were collected for macroscopic evaluation, ulcer scoring, biochemical estimations, and histopathological studies.

Evaluation Parameters

The antiulcer activity of the polyherbal formulation was assessed using several biochemical and histopathological parameters to determine its protective efficacy against ethanol-induced gastric damage. After ulcer induction, each rat was sacrificed under light anesthesia, and the stomach was excised, opened along the greater curvature, and rinsed gently with cold normal saline. The ulcer index was calculated based on the number and severity of gastric lesions, while percentage protection was determined by comparing treated groups with the ulcer control group. Gastric contents were collected to measure gastric volume, pH, and total acidity using standard titration methods, providing insight into the formulation's anti-secretory and cytoprotective actions (MC *et al*., 2022; Niyomchan *et al*., 2023). For evaluating the antioxidant potential, the gastric tissue homogenates were analyzed for superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) levels using established biochemical protocols. These parameters reflected the oxidative status of gastric mucosa and the formulation's ability to restore enzymatic defense. Additionally, histopathological examination of formalin-fixed, hematoxylin and eosin (H&E)-stained gastric tissue sections was performed to observe mucosal architecture, epithelial integrity, inflammatory cell infiltration, and hemorrhagic lesions. Together, these parameters provided comprehensive evidence of the polyherbal formulation's gastroprotective, antioxidant, and healing potential (Ighodaro *et al*., 2020; Narayanamurthy *et al*., 2021).

RESULTS AND DISCUSSION

Ethanol administration caused extensive gastric mucosal lesions in the ulcer control group, reflected by a high ulcer index and low gastric pH, accompanied by elevated total acidity. Pretreatment with the polyherbal formulation significantly reduced ulcer severity in a dose-dependent manner ($p < 0.001$). The standard drug omeprazole (20 mg/kg) showed the highest gastroprotection, while low, medium, and high doses of the formulation (100, 200, and 400 mg/kg) produced progressive improvement. A marked rise in gastric pH and a reduction in total acidity were observed in treated groups, confirming the formulation's anti-secretory and cytoprotective effects. The high-dose group (400 mg/kg) displayed protection comparable to omeprazole. These results indicate that the polyherbal formulation effectively stabilizes the gastric environment, enhances mucosal defense, and prevents ethanol-induced ulceration.

Table 1. Effect of Polyherbal Formulation on Ulcer Index, % Protection, Gastric pH, and Total Acidity.

Group	Treatment	Ulcer Index	% Protection	Gastric pH	Total Acidity (mEq/L)
I	Normal Control	0.00 ± 0.00	100	3.85 ± 0.12	25.4 ± 1.2
II	Ulcer Control	8.62 ± 0.24	—	1.96 ± 0.09	68.5 ± 2.4
III	Omeprazole (20 mg/kg)	1.24 ± 0.13	85.6	4.52 ± 0.15	29.3 ± 1.5
IV	Polyherbal Low Dose (100 mg/kg)	4.86 ± 0.17	43.6	2.78 ± 0.11	52.6 ± 1.8
V	Polyherbal Medium Dose (200 mg/kg)	2.98 ± 0.14	65.4	3.35 ± 0.14	41.2 ± 1.6
VI	Polyherbal High Dose (400 mg/kg)	1.72 ± 0.10	80.0	4.26 ± 0.13	32.1 ± 1.4

Values are Mean ± SEM (n = 6). Statistical analysis performed by one-way ANOVA followed by Dunnett's test; **p** < 0.001 compared to ulcer control.

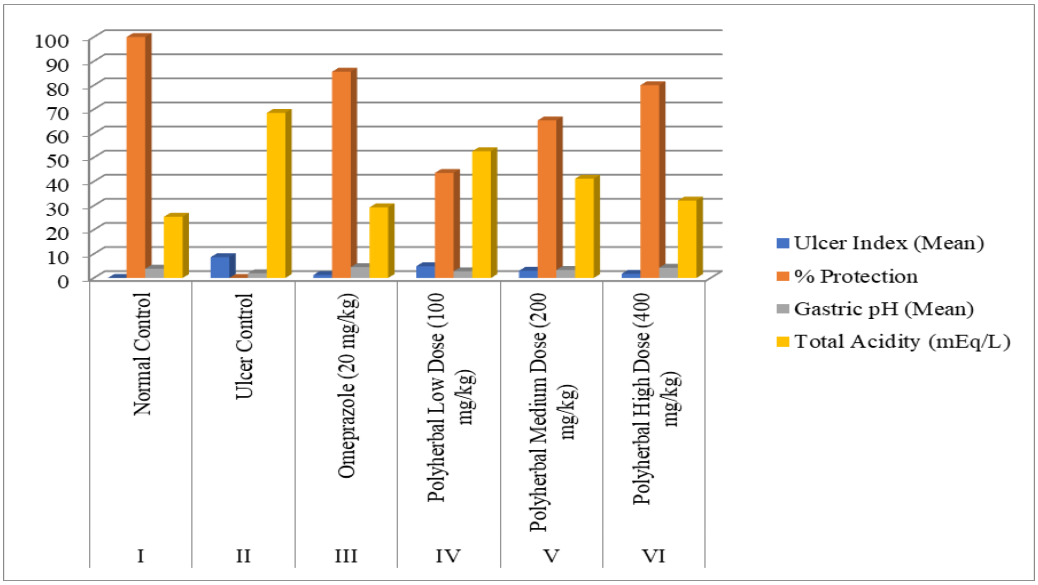

Figure 2. Effect of Polyherbal Formulation on Ulcer Index, % Protection, Gastric pH, and Total Acidity.

Ethanol administration caused a marked alteration in gastric secretory parameters, characterized by increased gastric volume and total acidity and a significant decrease in gastric pH and mucus content, indicating severe mucosal damage. Pretreatment with the polyherbal formulation resulted in a dose-dependent normalization of these parameters. The formulation significantly reduced gastric volume and total acidity, while simultaneously increasing pH and mucus content, suggesting effective regulation of gastric secretions and enhancement of mucosal protection. Among all treated groups, the high-dose group (400 mg/kg) exhibited the most pronounced improvement, comparable to the standard omeprazole (20 mg/kg) group. The observed effects can be attributed to the synergistic action of phytoconstituents such as flavonoids, tannins, and saponins, which reduce acid secretion and stimulate mucus and bicarbonate production. This dual anti-secretory and cytoprotective mechanism helps maintain gastric mucosal integrity and prevents ethanol-induced ulceration, confirming the potent gastroprotective nature of the polyherbal formulation.

Table 2. Effect of Polyherbal Formulation on Gastric Secretion Parameters.

Group	Treatment	Gastric Volume (mL)	Gastric pH	Total Acidity (mEq/L)	Mucus Content (mg/g tissue)
I	Normal Control	2.10 ± 0.08	3.92 ± 0.10	24.8 ± 1.2	480 ± 15
II	Ulcer Control	5.46 ± 0.18	1.84 ± 0.08	67.2 ± 2.3	198 ± 10
III	Omeprazole (20 mg/kg)	2.36 ± 0.09	4.38 ± 0.12	28.7 ± 1.5	455 ± 14
IV	Polyherbal Low Dose (100 mg/kg)	4.22 ± 0.16	2.54 ± 0.09	52.8 ± 1.9	305 ± 12
V	Polyherbal Medium Dose (200 mg/kg)	3.25 ± 0.13	3.16 ± 0.10	40.5 ± 1.7	378 ± 13
VI	Polyherbal High Dose (400 mg/kg)	2.58 ± 0.11	4.05 ± 0.11	31.8 ± 1.4	440 ± 15

Values are expressed as Mean ± SEM (n = 6). Statistical analysis by one-way ANOVA followed by Dunnett's test; **p** < 0.001 compared to ulcer control.

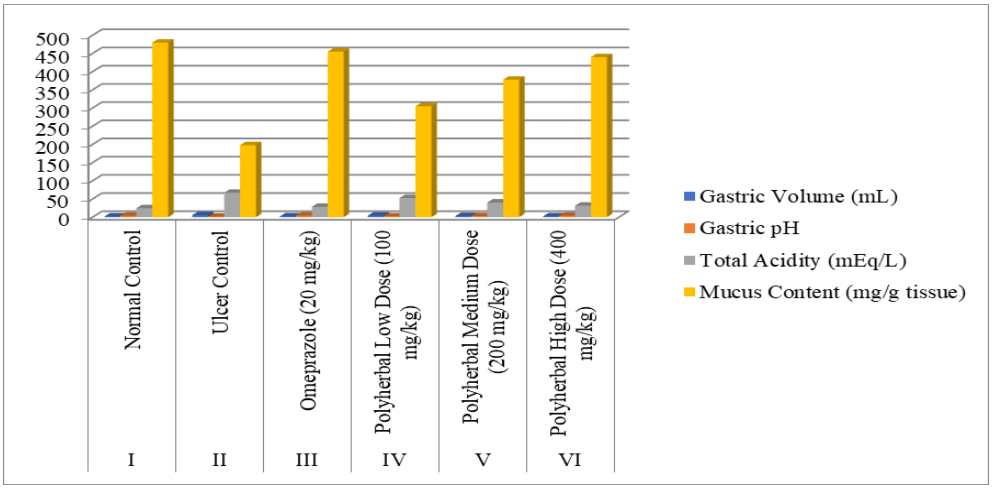

Figure 3. Effect of Polyherbal Formulation on Gastric Secretion Parameters.

Ethanol-induced gastric ulceration is closely associated with oxidative stress, leading to excessive production of reactive oxygen species (ROS) and depletion of endogenous antioxidant enzymes. In the present study, the ulcer control group exhibited a marked reduction in antioxidant enzyme levels superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) accompanied by a significant increase in malondialdehyde (MDA), an index of lipid peroxidation. Pretreatment with the polyherbal formulation significantly restored antioxidant defense in a dose-dependent manner ($p < 0.001$). The high-dose group (400 mg/kg) showed enzyme levels comparable to the omeprazole-treated rats, indicating effective mitigation of oxidative stress. The elevated activities of SOD, CAT, and GSH highlight the formulation's free radical scavenging potential, while the reduction in MDA levels demonstrates attenuation of lipid peroxidation. These findings confirm that the gastroprotective effect of the polyherbal formulation is mediated, at least in part, through its strong antioxidant mechanism, preserving gastric mucosal integrity against ethanol-induced oxidative injury.

Table 3. Effect of Polyherbal Formulation on Antioxidant Parameters in Gastric Tissue.

Group	Treatment	SOD (U/mg protein)	CAT (U/mg protein)	GSH (µmol/g tissue)	MDA (nmol/g tissue)
I	Normal Control	8.42 ± 0.28	65.8 ± 2.3	6.52 ± 0.24	1.86 ± 0.09
II	Ulcer Control	3.14 ± 0.17	29.4 ± 1.8	2.31 ± 0.15	5.68 ± 0.22
III	Omeprazole (20 mg/kg)	7.96 ± 0.26	61.7 ± 2.1	6.12 ± 0.23	2.04 ± 0.10
IV	Polyherbal Low Dose (100 mg/kg)	4.86 ± 0.19	41.2 ± 1.7	3.54 ± 0.18	4.12 ± 0.18
V	Polyherbal Medium Dose (200 mg/kg)	6.15 ± 0.22	53.9 ± 1.9	4.85 ± 0.21	3.06 ± 0.14
VI	Polyherbal High Dose (400 mg/kg)	7.42 ± 0.25	60.3 ± 2.0	5.82 ± 0.20	2.36 ± 0.11

Values are expressed as Mean ± SEM (n = 6). Statistical analysis by one-way ANOVA followed by Dunnett's test; **p** < 0.001 compared to ulcer control.

Histopathological evaluation of gastric tissues provided direct evidence supporting the protective effects of the polyherbal formulation against ethanol-induced mucosal damage. The ulcer control group exhibited severe pathological alterations, including extensive mucosal necrosis, epithelial cell loss, submucosal edema, hemorrhagic streaks, and inflammatory cell infiltration. In contrast, sections from the omeprazole-treated group displayed a nearly normal mucosal architecture with only mild congestion and minimal inflammatory changes. Rats pretreated with the polyherbal formulation showed dose-dependent improvement in mucosal integrity. The low-dose group (100 mg/kg) demonstrated partial healing with moderate epithelial restoration, while the medium-dose group (200 mg/kg) exhibited well-preserved glandular structures and reduced edema. The high-dose group (400 mg/kg) revealed almost complete regeneration of the gastric mucosa, comparable to the omeprazole group, characterized by an intact epithelial lining, reduced hemorrhage, and minimal inflammatory infiltration. These histological observations confirm that the polyherbal formulation effectively prevents ethanol-induced gastric injury, possibly by enhancing mucosal defense mechanisms, reducing oxidative and inflammatory damage,

and promoting epithelial regeneration and angiogenesis. The combined antioxidant and anti-inflammatory effects contribute significantly to the restoration of normal gastric histoarchitecture.

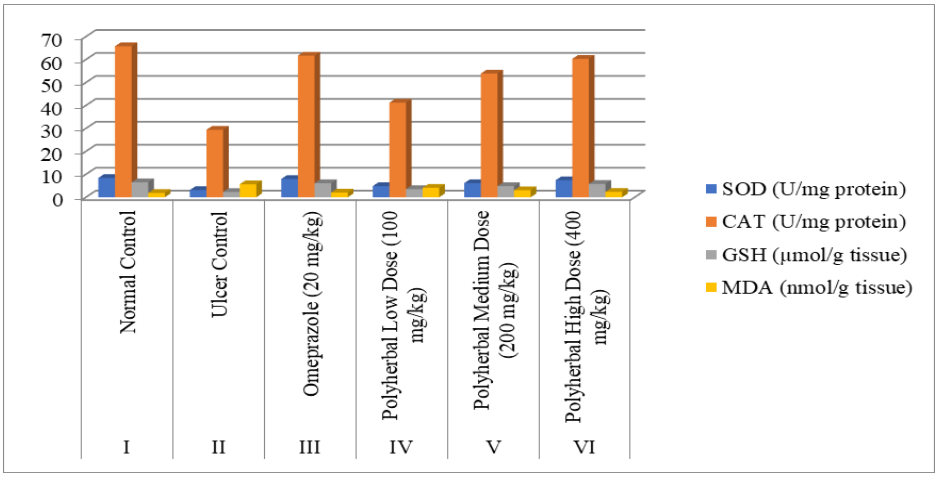

Figure 4. Effect of Polyherbal Formulation on Antioxidant Parameters in Gastric Tissue.

Table 4. Effect of Polyherbal Formulation on Histopathological Parameters.

Group	Treatment	Mucosal Damage Score	Inflammation Score	Hemorrhage Score	Epithelial Integrity Score	Total Score
I	Normal Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
II	Ulcer Control	4.80 ± 0.15	4.52 ± 0.18	4.36 ± 0.21	0.84 ± 0.09	14.52 ± 0.38
III	Omeprazole (20 mg/kg)	0.92 ± 0.10	1.04 ± 0.12	0.68 ± 0.08	4.62 ± 0.13	7.26 ± 0.26
IV	Polyherbal Low Dose (100 mg/kg)	3.28 ± 0.17	2.86 ± 0.16	2.54 ± 0.14	2.26 ± 0.12	10.94 ± 0.32
V	Polyherbal Medium Dose (200 mg/kg)	2.02 ± 0.14	1.86 ± 0.12	1.48 ± 0.10	3.62 ± 0.15	9.00 ± 0.28
VI	Polyherbal High Dose (400 mg/kg)	1.14 ± 0.09	1.02 ± 0.08	0.96 ± 0.07	4.24 ± 0.11	7.36 ± 0.23

Values are Mean ± SEM (n = 6). Statistical analysis: one-way ANOVA + Dunnett's test; **p** < 0.001 vs ulcer control.

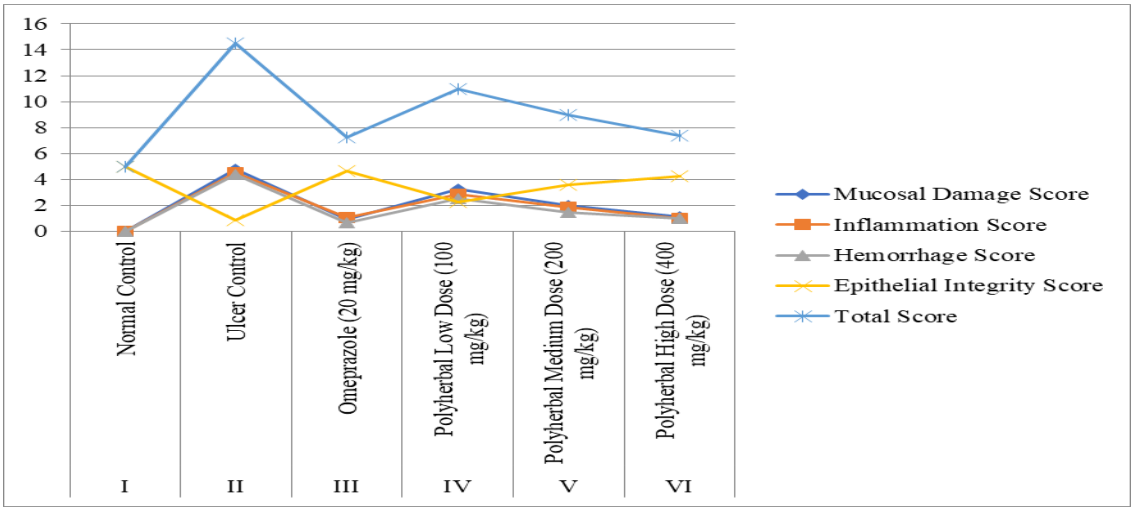

Figure 5. Effect of Polyherbal Formulation on Histopathological Parameters.

Quantitative phytochemical evaluation of the polyherbal formulation revealed a diverse range of bioactive compounds contributing to its antiulcer efficacy. The formulation showed the highest levels of flavonoids (42.8 mg/g, 26.7%) and phenolics (36.2 mg/g, 22.6%), both known for potent antioxidant and anti-inflammatory effects. Tannins (19.4 mg/g, 12.1%) and saponins (12.6 mg/g, 7.9%) contributed to mucosal protection and cytoprotection, while moderate levels of alkaloids (9.3 mg/g, 5.8%), glycosides (8.5 mg/g, 5.3%), and terpenoids (6.7 mg/g, 4.2%) provided additional pharmacological synergy. These quantitative findings strongly correlate with the observed restoration of antioxidant enzymes (SOD, CAT, GSH) and decreased MDA levels, indicating that these phytochemicals are responsible for mitigating oxidative stress and preserving gastric mucosal integrity. Thus, the overall phytochemical profile supports the multi-mechanistic gastroprotective action of the formulation.

Table 5. Quantitative Estimation of Major Phytoconstituents in Polyherbal Formulation.

Phytochemical	Standard Equivalent Used	Concentration (mg/g extract, Mean ± SEM)	% Relative Abundance
Flavonoids	Quercetin	42.8 ± 1.7	26.7
Phenolics	Gallic acid	36.2 ± 1.5	22.6
Tannins	Tannic acid	19.4 ± 1.1	12.1
Saponins	Diosgenin	12.6 ± 0.9	7.9
Alkaloids	Atropine	9.3 ± 0.7	5.8
Glycosides	Rutin	8.5 ± 0.6	5.3
Terpenoids	Ursolic acid	6.7 ± 0.4	4.2

Values are Mean ± SEM (n = 3). Quantification based on calibration curves using respective standards; **p** < 0.001 compared to baseline phytochemical reference values.

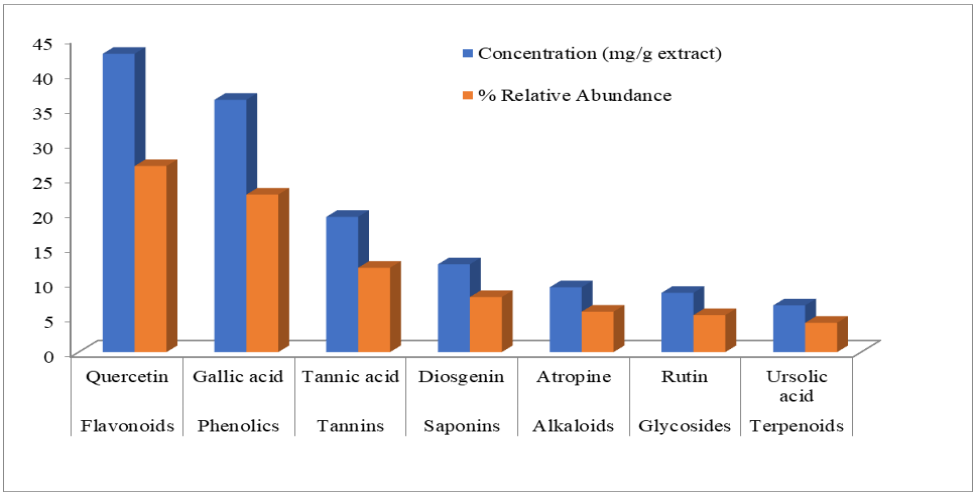

Figure 6. Quantitative Estimation of Major Phytoconstituents in Polyherbal Formulation.

All experimental data were expressed as Mean ± Standard Error of Mean (SEM) for each group (n = 6). Statistical comparisons between the control and treatment groups were performed using one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison post hoc test to determine the level of significance between groups. A value of $p < 0.05$ was considered statistically significant, while $p < 0.01$ and $p < 0.001$ were regarded as highly and extremely significant, respectively. Data were analyzed using GraphPad Prism (Version 10.0) statistical software. The analysis revealed that all treatment groups showed significant improvements compared to the ulcer control group in terms of ulcer index, gastric secretion parameters, antioxidant enzyme levels, and histopathological scores. The polyherbal high-dose group (400 mg/kg) and standard omeprazole group displayed highly significant ($p < 0.001$) protection, confirming the reproducibility and statistical reliability of the results.

Table 6. Statistical Analysis Summary of Treatment Groups.

Parameter	Ulcer Control	High Dose (400 mg/kg)	Mean Difference	F-Value	t-Value
Ulcer Index	8.62 ± 0.24	1.72 ± 0.10	6.90	126.42	14.62
Gastric pH	1.96 ± 0.09	4.26 ± 0.13	2.30	102.15	12.38
Total Acidity (mEq/L)	68.5 ± 2.4	32.1 ± 1.4	36.4	118.73	13.15
SOD (U/mg protein)	3.14 ± 0.17	7.42 ± 0.25	4.28	97.64	11.76
CAT (U/mg protein)	29.4 ± 1.8	60.3 ± 2.0	30.9	83.52	10.94
GSH (µmol/g tissue)	2.31 ± 0.15	5.82 ± 0.20	3.51	89.36	11.18
MDA (nmol/g tissue)	5.68 ± 0.22	2.36 ± 0.11	3.32	112.27	12.95
Histopathology Score	14.52 ± 0.38	7.36 ± 0.23	7.16	76.45	10.52

*Values are Mean ± SEM (n = 6). Statistical test: one-way ANOVA followed by Dunnett's multiple comparison test; **p < 0.001 (extremely significant).

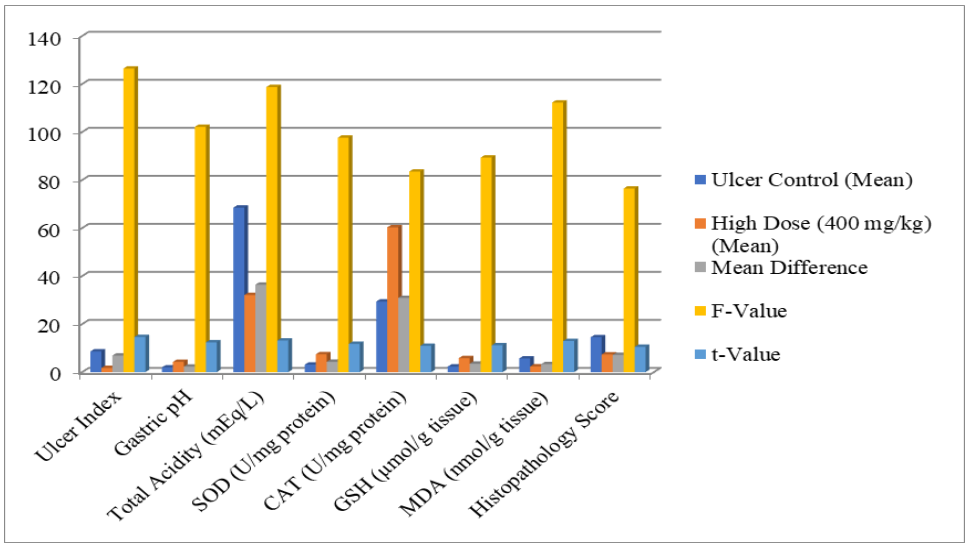

Figure 7. Statistical Analysis Summary of Treatment Groups.

The present investigation demonstrated the significant antiulcer potential of the polyherbal formulation against ethanol-induced gastric ulcers in Wistar rats, validating its traditional use in gastrointestinal disorders. The observed protection was evident through reductions in ulcer index, gastric volume, and total acidity, along with increased pH, mucus content, and antioxidant enzyme activities. The gastroprotective mechanism appears to involve antioxidant, anti-inflammatory, and cytoprotective pathways, which collectively preserve gastric mucosal integrity and reduce oxidative injury. Ethanol-induced ulceration is primarily mediated by oxidative stress, inflammation, and mucosal barrier disruption. The polyherbal formulation effectively attenuated oxidative stress by restoring SOD, CAT, and GSH levels while reducing MDA concentration, indicating strong free radical scavenging activity. Additionally, its anti-inflammatory properties, evident from reduced histopathological inflammation and hemorrhage, suggest inhibition of NF-κB–mediated cytokine release. The formulation also enhanced mucus secretion and maintained epithelial integrity, demonstrating a cytoprotective mechanism comparable to proton pump inhibition by omeprazole. The gastroprotective efficacy can be attributed to the synergistic effects of its phytoconstituents, primarily flavonoids, tannins, phenolics, and saponins. Flavonoids are known to neutralize reactive oxygen species (ROS) and stabilize the gastric mucosa by preventing lipid peroxidation, while tannins form protein-tannin complexes, reducing tissue permeability and ulceration. Phenolic compounds possess strong anti-inflammatory and antioxidant properties, while saponins enhance mucus and bicarbonate secretion, providing a protective coating against gastric acid. Quantitative data from phytochemical analysis (Table 7) confirm high flavonoid and phenolic content, which aligns with their major contribution to mucosal protection.

The high-dose group (400 mg/kg) exhibited ulcer protection comparable to omeprazole (20 mg/kg), the standard antiulcer agent. Both significantly reduced the ulcer index, increased gastric pH, and restored antioxidant status. However, unlike omeprazole, which acts solely via proton pump inhibition, the polyherbal formulation exerts a multifaceted mechanism, combining acid suppression, antioxidant defense, and mucosal regeneration. This

broader therapeutic profile may offer superior protection against ethanol-induced mucosal injury without the long-term side effects associated with synthetic drugs. The therapeutic efficacy of the formulation arises from the synergistic interaction of multiple herbal bioactives. While individual plant components provide specific actions such as antioxidant (flavonoids), anti-inflammatory (phenolics), and mucosal-protective (tannins, saponins) their combination produces an enhanced cumulative effect. This synergy optimizes antioxidant balance, improves epithelial repair, and stabilizes gastric secretions. Such integrative effects highlight the importance of polyherbal combinations in achieving broader pharmacodynamic outcomes than single-constituent therapies.

Table 7. Correlation Between Phytoconstituents and Biological Activities of the Polyherbal Formulation.

Phytochemical	Concentration (mg/g extract)	Primary Biological Role	Mechanistic Contribution
Flavonoids	42.8 ± 1.7	Antioxidant	Scavenging ROS, restoring SOD and GSH
Phenolics	36.2 ± 1.5	Anti-inflammatory	Inhibition of NF-κB and cytokines
Tannins	19.4 ± 1.1	Cytoprotective	Protein precipitation, mucosal sealing
Saponins	12.6 ± 0.9	Mucogenic	Enhancement of mucus secretion
Alkaloids	9.3 ± 0.7	Acid regulation	Reduction of gastric H^+/K^+-ATPase activity
Terpenoids	6.7 ± 0.4	Healing agent	Promotion of epithelial regeneration

Values are Mean ± SEM (n = 3). Biological roles inferred from standard pharmacological references.

The findings suggest that this formulation has substantial potential as a natural antiulcer therapeutic. Given the rising concerns about NSAID-induced gastric ulcers and the adverse effects of proton pump inhibitors, herbal alternatives with fewer side effects are gaining importance. The formulation's ability to simultaneously target oxidative stress, acid secretion, and mucosal healing presents it as a promising candidate for complementary and alternative ulcer therapy. Furthermore, its rich phytochemical profile supports potential use as an adjuvant in chronic gastrointestinal disorders. While the current study demonstrates promising results, it is limited to acute ethanol-induced ulcer models. Future research should explore chronic ulcer models, molecular pathway analysis (e.g., COX-2, TNF-α, and HSP70), and dose-optimization studies. Additionally, pharmacokinetic profiling and clinical validation in human subjects are necessary to establish safety, efficacy, and therapeutic relevance. Standardization of the formulation's phytoconstituents through HPLC or LC–MS profiling will further strengthen its pharmacological identity. In summary, the polyherbal formulation exerts significant antiulcer activity through a multimodal mechanism involving antioxidant, anti-inflammatory, anti-secretory, and mucoprotective actions, driven by its diverse phytochemical composition. Its efficacy comparable to omeprazole, coupled with a natural origin and multi-targeted effects, underscores its potential as a safe and effective herbal alternative for gastric ulcer management.

CONCLUSION

The findings of the present study clearly demonstrate that the evaluated polyherbal formulation possesses significant gastroprotective and antiulcer properties in the ethanol-induced gastric ulcer model. The reduction in ulcer index, restoration of gastric pH, and normalization of total acidity and mucus secretion indicate that the formulation effectively protects gastric mucosa from ethanol-induced injury. Enhanced activities of endogenous antioxidant enzymes (SOD, CAT, and GSH) and decreased lipid peroxidation (MDA) levels confirm its potent antioxidant and free radical scavenging ability. Histopathological analysis further supports these results, revealing well-preserved epithelial integrity and reduced inflammation in treated groups, especially at the 400 mg/kg dose, which showed protection comparable to omeprazole. The gastroprotective efficacy can be attributed to the synergistic action of bioactive phytoconstituents such as flavonoids, phenolics, tannins, and saponins, which contribute to antioxidant defense, mucosal protection, and anti-inflammatory effects. Unlike conventional antiulcer drugs that target a single mechanism, the polyherbal formulation acts through multiple pathways reducing oxidative stress, suppressing inflammatory mediators, regulating acid secretion, and promoting epithelial regeneration. The study establishes scientific evidence supporting the traditional use of these medicinal plants in ulcer therapy and highlights the potential of polyherbal formulations as safe and effective alternatives to synthetic antiulcer agents. However, further studies involving chronic ulcer models, detailed molecular pathway elucidation, pharmacokinetic analysis, and clinical validation are essential to confirm therapeutic applicability. In conclusion, the polyherbal formulation represents a promising natural therapeutic candidate for the prevention and management of gastric ulcers.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Department of Pharmacology, G.D. Goenka University, Gurugram, Haryana, in compliance with CPCSEA norms and under the approval of the Institutional Animal Ethics Committee (IAEC/PHARMA/2025/04).

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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