RESEARCH PAPER

Anti-Ulcer Potential of Polyherbal Formulation of Ficus benghalensis and Cynodon dactylon in Rats

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ABSTRACT

Background

The present study evaluated the acute oral toxicity and anti-ulcer potential of polyherbal formulations comprising Ficus benghalensis and Cynodon dactylon in rat models.

Methods

Behavioral observations and body weight monitoring revealed no treatment related adverse effects at doses of 300 mg/kg and 2000 mg/kg, with steady weight gain across all groups. Biochemical analyses confirmed the absence of significant alterations in glucose metabolism, lipid profile, renal, or hepatic function, supporting the safety of the formulations under acute exposure.

Results

In indomethacin and pyloric ligation induced ulcer models, the formulations demonstrated varying degrees of gastroprotection. Sample B consistently exhibited the strongest efficacy, significantly reducing ulcer scores, gastric volume, pepsin activity, and malondialdehyde levels, while enhancing mucin content and superoxide dismutase activity, approximating the protective effects of ranitidine. Sample A showed moderate activity, whereas Sample C was less effective.

Conclusion

Overall, the findings highlight the safety and therapeutic promise of these polyherbal formulations, with Sample B emerging as the most potent candidate for further pharmacological development against peptic ulcer disease.

KEYWORDS: Ficus benghalensis and Cynodon dactylon, polyherbal, gastroprotection, Ulcerogenic.

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INTRODUCTION

Peptic ulcer disease (PUD) remains one of the most prevalent gastrointestinal disorders worldwide, characterized by mucosal erosions in the stomach or duodenum due to an imbalance between aggressive factors such as gastric acid, pepsin, and *Helicobacter pylori* infection, and defensive mechanisms including mucus secretion, bicarbonate production, and mucosal blood flow[1]. Despite the availability of synthetic drugs like proton pump inhibitors, H_2-receptor antagonists, and antacids, their long-term use is often associated with adverse effects, drug resistance, and recurrence of ulcers. This has prompted a growing interest in natural remedies, particularly herbal formulations, which are perceived to be safer, cost-effective, and capable of providing holistic healing[2].

Polyherbalism, a cornerstone of traditional medicine systems such as Ayurveda, emphasizes the synergistic use of multiple plant species to enhance therapeutic efficacy and minimize toxicity[3]. Unlike single-herb preparations, polyherbal formulations combine bioactive compounds that may act on different pathways, thereby offering a broader spectrum of pharmacological activity. In the context of ulcer management, such formulations can simultaneously reduce gastric acid secretion, strengthen mucosal defenses, and exhibit antioxidant and anti-inflammatory properties[4].

Ficus benghalensis, commonly known as the banyan tree, has been revered in Indian traditional medicine for centuries. Its bark, aerial roots, and leaves are documented to possess anti-inflammatory, antioxidant, and wound-healing properties[5]. Phytochemical studies reveal the presence of flavonoids, tannins, and triterpenoids, which contribute to its gastroprotective potential. Tannins, in particular, are known to precipitate proteins and form a protective layer over the gastric

mucosa, thereby shielding it from irritants and excessive acid[6].

Cynodon dactylon, popularly called Bermuda grass, is another plant widely used in folk medicine[7]. Traditionally employed for its anti-inflammatory, antimicrobial, and immunomodulatory effects, it contains alkaloids, glycosides, and phenolic compounds that may play a role in ulcer healing. Its antioxidant activity helps neutralize free radicals, which are implicated in mucosal injury and delayed ulcer healing[8]. Moreover, its soothing effect on the gastrointestinal tract makes it a promising candidate for inclusion in anti-ulcer formulations[9].

The combination of *Ficus benghalensis* and *Cynodon dactylon* in a polyherbal formulation is based on the principle of synergism. While *Ficus benghalensis* primarily strengthens mucosal defenses and provides a protective barrier, *Cynodon dactylon* complements this by reducing oxidative stress and inflammation. Together, they may offer a dual mechanism of action: suppression of aggressive ulcerogenic factors and enhancement of defensive mucosal mechanisms. Such synergy not only improves therapeutic outcomes but also reduces the required dosage of individual herbs, thereby minimizing potential toxicity[10].

Animal models, particularly rats, are widely used to evaluate the anti-ulcer potential of herbal formulations. Induction of ulcers in rats can be achieved through various methods such as pylorus ligation, ethanol administration, or stress models, each mimicking different aspects of human ulcer pathology. These models allow researchers to assess parameters like ulcer index, gastric volume, acidity, and histopathological changes in the gastric mucosa. By testing the polyherbal formulation in such models, the efficacy and safety of the combination can be scientifically validated[11].

The exploration of polyherbal formulations for ulcer management represents a promising frontier in natural product research. By combining the gastroprotective properties of *Ficus benghalensis* with the antioxidant and anti-inflammatory effects of *Cynodon dactylon*, this study aims to provide scientific evidence for their synergistic efficacy in rat models. Ultimately, such research could contribute to the development of safe, effective, and affordable herbal therapies for peptic ulcer disease, addressing a global health concern with roots in traditional wisdom.

MATERIALS AND METHODS

Collection of plant: The leaves of *Ficus benghalensis* and *Cynodon dactylon* were locally collected from Rajasthan during the month of February. After collection, they were carefully separated, thoroughly washed with tap water to remove impurities, and subsequently shade-dried to preserve their phytoconstituents.

Authentication of plant: The collected plant specimens were authenticated by the Head of Office, Botanical Survey of India, Ministry of Environment, Forest and Climate Change, Jodhpur, Rajasthan (Ref. No. A.12012/Tech./2024-25(Pl.Id.)/525 12-B/834/2025, dated 06.11.2025). Authentication was carried out by comparing the morphological characteristics of the crude drug samples with standard descriptions.

***In-vivo* animal experimental study:**

Acute oral toxicity study

During the assessment for the acute toxicity study, all the animals were carefully monitored for any signs and symptoms of the development of toxicity at the first 30 min, 1 h and 24 h. They were continued every day for 14 days. The animal body weights were monitored daily, and the mean body weight was calculated for each week. In a preliminary study with a dose of 2000 mg/kg, there were no indications of toxicity. The body weight of each rat before receiving polyherbal formulation, after 7 days, and after 14 days was compared, and their graphs showed no significant difference ($p < 0.05$) in body weight. The weight gain for the week 1 and 2 during the 14-day observations was also analyzed and showed no prominent changes. The behavioral parameters of the female Wistar rat during the observational period were recorded in table where the animals showed normal signs after the dose. However, the water consumption was less in three animals when compared with the control group[12].

Study design: Three animals will be selected for each group. Three polyherbal formulations were prepared by the alcoholic extract of *F. benghalensis* and *C. dactylon*.

- **Test sample A: (25:75)** ethanolic extract of *F. benghalensis* and *C.dactylon* (200 mg/Kg body weight)
- **Test sample B: (50:50)** ethanolic extract of *F. benghalensis* and *C. dactylon* (200 mg/Kg body weight)
- **Test sample C: (75:25)** ethanolic extract of *F. benghalensis* and *C. dactylon* ((200 mg/Kg body weight)

Behavioral changes: Clinical evaluations were conducted on all animals outside their home cages once prior to the initial dose, then weekly thereafter, and again before necropsy. Observations focused on potential changes in skin, fur, eyes, and mucous membranes, as well as the presence of secretions or excretions. Additional signs monitored included lacrimation, piloerection, pupil size, respiratory patterns, alterations in gait or posture, responses to handling, excessive grooming, circling, and abnormal behaviors such as self-mutilation or walking backwards.

Body Weight, Food, and Water Consumption: The body weight of each rat was recorded weekly

and again immediately prior to necropsy. Food and water intake were also measured on a weekly basis, with results expressed as mean ± standard deviation (SD).

Biochemical changes:

Collection of Blood Samples and Serum Separation: At the conclusion of the experiment, rats were fasted for 24 hours, anesthetized with isoflurane, and euthanized by cervical dislocation. Blood samples were collected via intracardiac puncture. Serum was separated from the blood by centrifugation at 4 °C at 6,000 rpm for 15 minutes. The resulting serum was stored at –20 °C until biochemical analyses were performed[13].

Blood samples were collected into sterile tubes without anticoagulant for serum preparation and subsequent biochemical analysis. Parameters measured included alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, creatinine, albumin, total serum protein, urea, cholesterol, and triglycerides. Analyses were performed using an automated chemistry analyzer (Roche Diagnostics, IN, United States) following established protocols[14].

Indomethacin induced Ulcer model:

In brief, rats were treated with a single oral dose of indomethacin (30 mg/kg body weight). They were deprived of food but had free access to water 24h prior to ulcer induction. The animals are divided into six groups having six animals in each group. Group-1 received normal saline (0.9 % NaCl). Group-2 will serve as positive control that received indomethacin. Group-3 will receive indomethacin as an Ulcerogen and standard drug such as Ranitidine (30 mg/kg body weight). Treatment with the reference drug and test sample will last for 21 days prior to indomethacin administration[15].

Pylorus Ligation induced model:

In brief, rats were treated with a single oral dose of indomethacin (30mg/kg body weight). They were deprived of food but had free access to water 24h prior to ulcer induction. The animals are divided into six groups having six animals in each group.

The rats were deprived of food but had free access to water for 1 day before pyloric ligation was carried out, pylorus ligation was performed in the animals for the induction of gastric ulcers 1 hour after the last administration of rapidly different test solutions on fasted rats. The abdomen was opened by using a small incision below the xiploid process after induction of anesthesia by ketamine hydrochloride (150 mg/kg, Intraperitoneal) and xylazine (10 mg/kg). The stomach was exposed and a thread was placed around the pyloric sphincter and tied in a tight knot. The stomach was placed back carefully and the abdominal wall was closed with sutures. The animals were deprived of food and water during the postoperative period and the animals were sacrificed 5 hours after pylorus ligation by overdose of xylazine and ketamine. The stomachs were removed and the contents were drained into tubes and centrifuged at 1000 rpm for 10 minutes. The supernatant was then subjected to analysis for gastric volume, gastric PH, free acidity, total acidity and pepsin content according[16].

Animal grouping and treatments:

Group 1: Control group (Receive normal saline, no treatment, n=6)
Group 2: Negative control (ulcer induced) treated with Ranitidine (30 mg/kg) drug, n=6)
Group 3: Positive control (animal (indomethacin (30 mg/kg)), n=6)
Group 4: Test Control (animal (ulcer induced) treated with test sample in A part, n=6)
Group 5: Test Control (animal (ulcer induced) treated with test sample in B part, n=6)
Group 6: Test Control (animal (ulcer induced) treated with test sample in C part, n=6)

Observation:

- Assessment of ulcer score, ulcer index and percentage ulcer inhibition.
- Gastric volume and pH
- Effect on pepsin activity and mucin content
- Assessment of SOD (Superoxide dismutase) in stomach homogenate
- Assessment of level of lipid peroxidation in stomach homogenate

RESULTS AND DISCUSSION

Acute oral toxicity study

Behavioral changes: Observable parameters and weight changes in different dose groups are mentioned in Tables. No changes related to treatment have been observed. A soft fecal consistency was observed in all rats in the treatment groups; however, this symptom was not observed on 2nd day after administration. No changes were observed after gross necroscopy of both compounds (data not shown). As per observations, it was assumed that unclassified compound which might show toxicity at 2000 mg/kg.

Body weight: Administration of the polyherbal formulation at both 300 mg/kg and 2000 mg/kg doses did not produce any adverse effect on the growth pattern of rats over the 14-day observation period. In all three samples (A, B, and C), a gradual increase in body weight was observed from Day 0 to Day 14, indicating normal physiological development and absence of toxicity. For instance, rats treated with Sample A at 300 mg/kg showed an increase from 146.8 ± 2.1 g on Day 0 to 157.3 ± 1.6 g on Day 14, while those receiving the higher dose of 2000 mg/kg exhibited a similar upward trend (153.5 ± 1.7 g to 160.3 ± 2.1 g). Comparable

patterns were noted in Samples B and C, where body weights consistently increased across the study period, even at the higher dose levels. The steady gain in weight across all groups suggests that the polyherbal formulation is well tolerated and does not interfere with normal metabolic processes. These findings support the safety profile of the formulation under acute oral toxicity conditions, reinforcing its potential for further pharmacological evaluation.

Table No. 1: Body weight variation for acute oral toxicity

Dose of poly-herbal Formulation (mg)	Body weight (gm)		
	Day 0	Day 7	Day 14
Sample A 300 mg/kg	146.8 ± 2.1	152.4 ± 3.1	157.3 ± 1.6
Sample A 2000 mg/kg	153.5 ± 1.7	156.3 ± 2.1	160.3 ± 2.1
Sample B 300 mg/kg	149.6 ± 2.6	154.6 ± 1.9	157.5 ± 3.1
Sample B 2000 mg/kg	137.8 ± 3.5	143.7 ± 2.3	148.4 ± 2.1
Sample C 300 mg/kg	138.6 ± 2.3	144.7 ± 1.7	151.3 ± 4.2
Sample C 2000 mg/kg	132.2 ± 3.1	138.4 ± 2.9	142.8 ± 3.4

Biochemical changes: Biochemical analysis result values are presented in Table. The acute oral toxicity study was conducted to evaluate the safety profile of the test formulations by monitoring biochemical parameters across different groups. The results presented in Table indicated that administration of the polyherbal formulations did not produce any significant adverse biochemical alterations compared to the control group.

The biochemical parameters collectively demonstrate that acute oral administration of polyherbal formulations did not produce toxic effects on glucose metabolism, lipid profile, renal function, or hepatic function. The absence of significant deviations from control values supports the safety of these formulations under acute exposure conditions.

Table No. 2: Biochemical changes for acute oral toxicity

Behavior	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI
Glucose mg/dL	88.21 ± 9.32	85.27 ± 9.71	90.42 ± 8.55	92.16 ± 6.37	87.63 ± 4.93	89.72 ± 9.28
Cholesterol mMol/L	78.34 ± 4.73	75.13 ± 3.11	77.63 ± 5.32	74.39 ± 8.4	78.67 ± 4.7	72.69 ± 7.2
Triglycerides mMol/L	83.72 ± 3.87	87.63 ± 4.73	84.26 ± 5.33	81.63 ± 3.83	88.57 ± 7.52	89.63 ± 7.53
Urea mg/dL	28.73 ± 2.73	30.86 ± 3.27	34.75 ± 2.83	37.74 ± 1.94	31.83 ± 2.77	35.18 ± 4.11
Albumin mg/dL	3.61 ± 0.12	3.86 ± 0.64	3.35 ± 0.74	3.89 ± 0.81	3.78 ± 0.73	3.64 ± 0.27
BUN mg/dL	14.83 ± 1.82	15.82 ± 0.95	14.21 ± 0.85	15.38 ± 0.63	14.88 ± 0.72	15.17 ± 0.83
Creatinine mg/dL	0.87 ± 0.16	0.79 ± 0.13	0.88 ± 0.17	0.72 ± 0.21	0.83 ± 0.32	0.78 ± 0.71
Protein mg/dL	6.98 ± 0.88	6.87 ± 0.62	6.89 ± 0.77	6.42 ± 0.46	6.74 ± 0.17	6.28 ± 0.42
Bilirubin mg/mL	3.84 ± 0.37	3.47 ± 0.82	3.56 ± 0.17	3.18 ± 0.52	3.56 ± 0.84	3.18 ± 0.63
ALP U/L	227.23 ± 10.28	238.38 ± 12.62	217.82 ± 11.28	217.28 ± 14.29	220.42 ± 13.52	227.61 ± 11.32
AST U/L	124.22 ± 4.21	134.78 ± 3.12	146.81 ± 2.73	134.32 ± 4.31	141.28 ± 5.11	137.28 ± 4.12
ALT U/L	29.33 ± 2.12	28.12 ± 1.82	27.73 ± 1.72	26.28 ± 1.28	29.28 ± 0.95	28.27 ± 0.89

Indomethacin induced Ulcer model

Among the treated groups exhibited varying degrees of protection against indomethacin-induced damage. The mucosal surfaces in these groups show fewer lesions and improved tissue appearance compared to the positive control, suggesting that the polyherbal formulations confer gastroprotective effects. The reduction in ulcer severity across these samples highlights their potential in mitigating ulcerogenic injury, with visible improvements comparable to the standard drug treatment. Overall, the figure provides clear visual evidence that the polyherbal formulations of *Ficus benghalensis* and *Cynodon dactylon* contribute to the preservation of gastric mucosal integrity, supporting their anti-ulcer efficacy in experimental models.

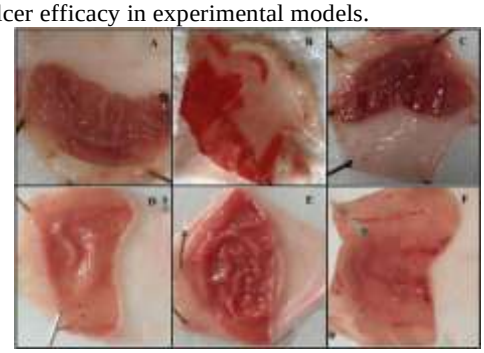


Figure 1: Photographs of indomethacin induced animals (A) Normal control (B) Positive control (Indomethacin treated) (C) Negative control

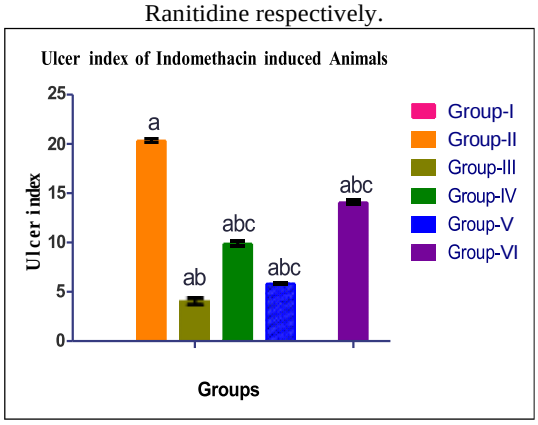
(Ranitidine treated) (D) Treated with sample A (E) Treated with sample B (F) Treated with sample C.

Quantification of Ulceration: Indomethacin administration in Group II (positive control) produced a marked increase in ulcer index (20.32 ± 0.45), confirming its strong ulcerogenic potential. Group III animals treated with ranitidine (negative control) showed a significant reduction in ulcer score (4.01 ± 0.83) with 80.27% inhibition, validating the protective role of Among the test formulations, Sample B (Group V) demonstrated the most promising anti-ulcer activity, with an ulcer score of 5.83 ± 0.13 and 71.31% inhibition. Sample A (Group IV) showed moderate protection, reducing the ulcer score to 9.87 ± 0.57 with 51.43% inhibition. This indicates partial efficacy, which may be dose-dependent or related to the specific phytochemical composition. In contrast, Sample C (Group VI) exhibited the least protective effect, with an ulcer score of 14.07 ± 0.52 and only 30.76% inhibition, suggesting limited therapeutic potential at the tested dose. Overall, the results highlight that poly-herbal formulations can exert varying degrees of gastroprotection, with Sample B showing the highest efficacy.

Table No. : Ulcer score and % ulcer inhibition in Indomethacin induced Animals

Groups	Poly-herbal Formulation	Ulcer Score	Ulcer index	% Ulcer Inhibition	Gastric volume (ml)	Gastric pH
Group-I	Control	0	-	-	2.06 ± 0.51	6.80 ± 0.11
Group-II	Positive control (indomethacin)	5	20.32 ± 0.45[a]	-	9.54 ± 0.47 [a]	2.20 ± 0.74 [a,]
Group-III	Negative control	1	4.01 ± 0.83[a, b]	80.27	2.89 ± 0.23 [b]	5.60 ± 0.16 [a, b]
Group-IV	Sample A	2	9.87 ± 0.57 [a, b, c]	51.43	5.74 ± 0.64 [a, b, c]	3.20 ± 0.12 [a, b, c]
Group-V	Sample B	1	5.83 ± 0.13 [a, b, c]	71.31	3.21 ± 0.78 [a, b]	2.80 ± 0.31 [a, c]
Group-VI	Sample C	3	14.07 ± 0.5	30.76	7.03 ± 0.62 [a, b, c]	4.40 ± 0.62 [a, b, c]

Values were presented as means of 6 mice ± SEM.[a,b,c] significantly different (***$p < 0.0001$) from Control group, Indomethacin (IND), and Ranitidine respectively.



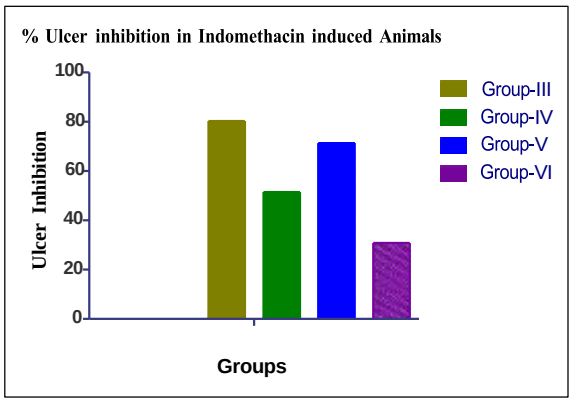
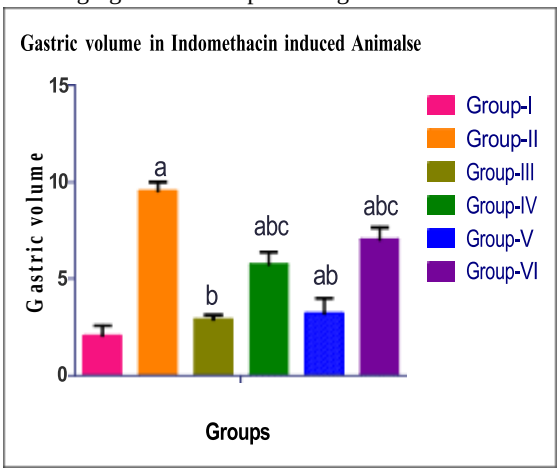


Figure: Ulcer index and % Ulcer Inhibition of Indomethacin induced Animals

Values were presented as means of 6 mice ± SEM.[a,b,c] significantly different (***$p < 0.0001$) from Control group, Indomethacin (IND), and Ranitidine respectively.

Gastric volume and pH in Indomethacin induced Animals: Indomethacin administration (Group II) markedly increased gastric volume (9.54 ± 0.47 mL) while significantly lowering gastric pH (2.20 ± 0.74), In contrast, ranitidine treatment (Group III) effectively reduced gastric volume (2.89 ± 0.23 mL) and maintained a near-neutral pH (5.60 ± 0.16). Among the test formulations, Sample B (Group V) showed the most favorable results, with a gastric volume of 3.21 ± 0.78 mL and a moderately acidic pH of 2.80 ± 0.31. Although the pH remained lower than that of the ranitidine group, the reduced gastric volume suggests a protective effect against excessive acid secretion. Sample A (Group IV) demonstrated partial efficacy, lowering gastric volume to 5.74 ± 0.64 mL and raising pH to 3.20 ± 0.12, indicating moderate gastroprotection. Sample C (Group VI) showed limited

effectiveness, with a relatively high gastric volume (7.03 ± 0.62 mL) and intermediate pH (4.40 ± 0.62), suggesting weaker anti-secretory activity compared to the other formulations. Overall, these findings highlight that poly-herbal formulations can modulate gastric secretory parameters, with Sample B emerging as the most promising candidate.



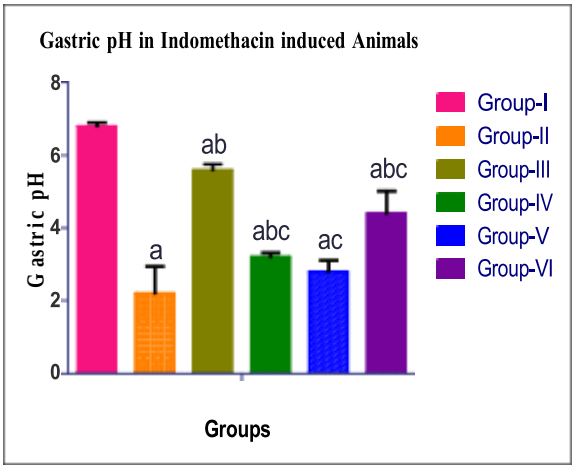


Figure: Gastric volume and Gastric pH in Indomethacin induced Animals

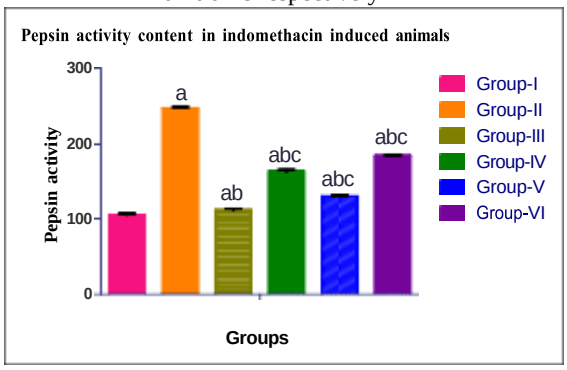
Values were presented as means of 6 mice ± SEM.[a,b,c] significantly different (***$p < 0.0001$) from Control group, Indomethacin (IND), and Ranitidine respectively.

Pepsin activity and mucin content in Indomethacin induced Animals: Indomethacin treatment (Group II) markedly elevated pepsin activity (247.71 ± 1.34 µg/mL) while significantly reducing mucin content (183.73 ± 2.14 µg/mL). In contrast, ranitidine (Group III) maintained pepsin activity close to normal levels (112.17 ± 1.72 µg/mL) and preserved mucin content (387.97 ± 3.42 µg/mL). Among the test formulations, Sample B (Group V) showed the most favorable outcome, with pepsin activity reduced to 130.31 ± 1.21 µg/mL and mucin content elevated to 357.25 ± 3.22 µg/mL. Sample A (Group IV) demonstrated moderate efficacy, lowering pepsin activity to 163.14 ± 2.15 µg/mL and increasing mucin to 307.62 ± 2.17 µg/mL, indicating partial protection. Sample C (Group VI**)** was less effective, with relatively high pepsin activity (184.27 ± 0.89 µg/mL) and reduced mucin levels (234.81 ± 2.65 µg/mL), reflecting weaker anti-ulcer potential.

Table No. : Pepsin activity and mucin content in Indomethacin induced Animals

Groups	**Poly-herbal Formulation**	**Pepsin activity (µg/ml)**	**Mucin content (µg/ml)**	**SOD Activity µg/mg**	**MDA Conc. µmol/mg**
Group-I	Control	105.32 ± 2.14	401.63 ± 1.43	189.34 ± 1.32	2.20 ± 0.03
Group-II	Positive control (indomethacin)	247.71 ± 1.34 [a]	183.73 ± 2.14 [a]	97.71 ± 0.74[a]	6.40 ± 0.04[a]
Group-III	Negative control (Ranitidine)	112.17 ± 1.72 [a, b]	387.97 ± 3.42 [a, b]	170.26 ± 1.28 [a, b]	2.50 ± 0.02[a, b]
Group-IV	Sample in A	163.14 ± 2.15 [a, b, c]	307.62 ± 2.17 [a, b, c]	146.93 ± 0.36 [a, b, c]	2.90 ± 0.05[a, b, c]
Group-V	Sample in B	130.31 ± 1.21 [a, b, c]	357.25 ± 3.22 [a, b, c]	161.81 ± 0.52 [a, b, c]	2.60 ± 0.06[a, b, c]
Group-VI	Sample in C	184.27 ± 0.89 [a, b, c]	234.81 ± 2.65 [a, b, c]	132.28 ± 0.88 [a, b, c]	3.30 ± 0.02[a, b, c]

Values were presented as means of 6 mice ± SEM.[a, b, c] significantly different (***$p < 0.0001$) from Control group, Indomethacin (IND), and Ranitidine respectively



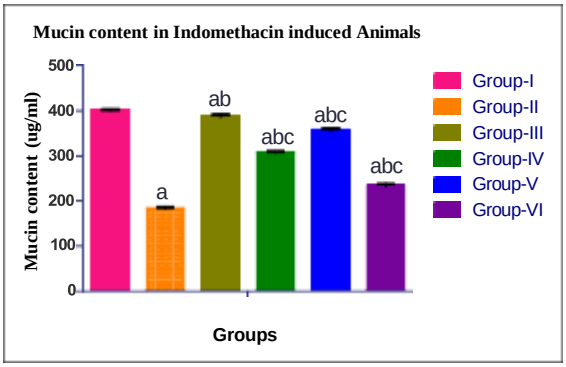
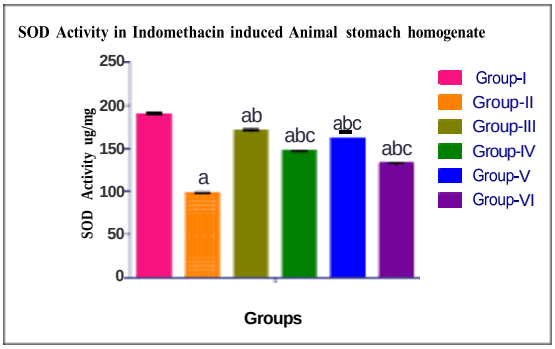


Figure : Pepsin activity and Mucin content in indomethacin induced animals

Values were presented as means of 6 mice ± SEM.[a,b,c] significantly different (***$p < 0.0001$) from Control group, Indomethacin (IND), and Ranitidine respectively

Assessment of stomach homogenate in Indomethacin induced Animals: Indomethacin administration (Group II) resulted in a significant decrease in superoxide dismutase (SOD) activity (97.71 ± 0.74 µg/mg protein) and a marked increase in malondialdehyde (MDA) concentration (6.40 ± 0.04 µmol/mg protein). In contrast, ranitidine treatment (Group III) preserved antioxidant status, maintaining relatively high SOD activity (170.26 ± 1.28 µg/mg protein) and low MDA levels (2.50 ± 0.02 µmol/mg protein). Among the poly-herbal formulations, Sample B (Group V) demonstrated the strongest antioxidant potential, with SOD activity elevated to 161.81 ± 0.52 µg/mg protein and MDA reduced to 2.60 ± 0.06 µmol/mg protein. Sample A (Group IV) showed moderate efficacy, with SOD activity at 146.93 ± 0.36 µg/mg protein and MDA at 2.90 ± 0.05 µmol/mg protein, indicating partial protection. Sample C (Group VI) was less effective, with lower SOD activity (132.28 ± 0.88 µg/mg protein) and higher MDA levels (3.30 ± 0.02 µmol/mg protein), reflecting weaker antioxidant defense.



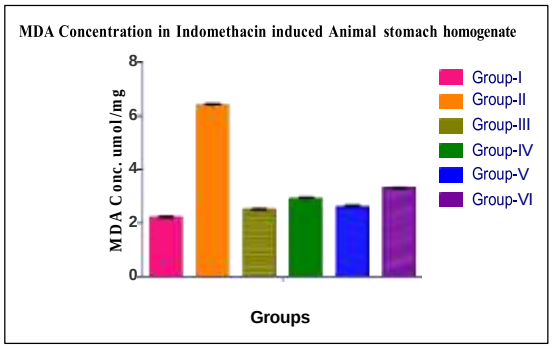


Figure : SOD Activity and MDA Conc. in Indomethacin induced animal homogenate

Values were presented as means of 6 mice ± SEM.[a,b,c] significantly different (***$p < 0.0001$) from Control group, Indomethacin (IND), and Ranitidine respectively

Histopathology

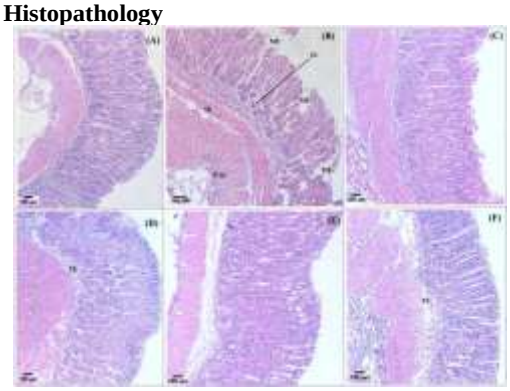


Figure : The Histopathological changes were assessed in indomethacin induced animals (A) Normal control (B) Positive control (pyloric ligation induced) (C) Negative control (Ranitidine treated) (D) Treated with sample A (E) Treated with sample B (F) Treated with sample C. Where epithelial cell loss (EL), hemorrhage (H), inflammatory cell (leucocytes) infiltration and edema (IE) and mucosal erosions (ME)

Pyloric Ligation induced Ulcer model

In pyloric ligation–induced ulcer models, the photographs clearly demonstrate mucosal differences across groups. The normal control shows intact gastric lining, while the positive control exhibits severe ulceration. Ranitidine treatment reduces lesions, and samples A, B, and C display notable protection, confirming the polyherbal formulation's anti-ulcer efficacy and safety.

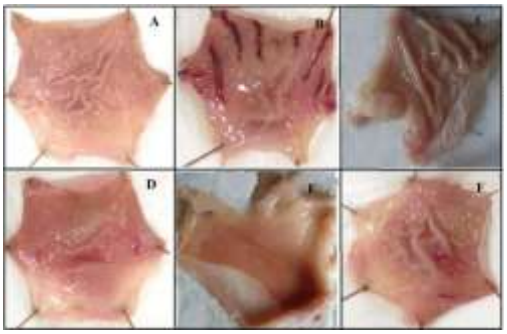


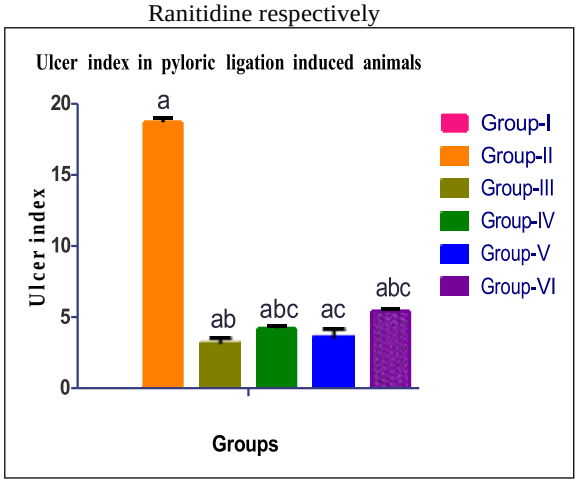
Figure : Photographs of Pyloric ligation induced animals (A) Normal control (B) Positive control (pyloric ligation induced) (C) Negative control (Ranitidine treated) (D) Treated with sample A (E) Treated with sample B (F) Treated with sample C.

Ulcer score and percentage ulcer inhibition: Pyloric ligation in Group II (positive control) produced a significant increase in ulcer score (18.72 ± 0.28). Ranitidine (Group III) served as the standard reference drug, showing a marked reduction in ulcer score (3.17 ± 0.32) with 83.07% inhibition. Among the poly-herbal formulations, Sample B (Group V) demonstrated the most promising gastroprotective effect, with an ulcer score of 3.58 ± 0.53 and 80.88% inhibition, closely approximating the efficacy of ranitidine. Sample A (Group IV**)** also showed substantial protection, reducing the ulcer score to 4.21 ± 0.17 with 77.51% inhibition. In contrast, Sample C (Group VI) exhibited weaker activity, with a higher ulcer score (5.42 ± 0.18) and lower inhibition (71.05%), suggesting limited therapeutic potential at the tested dose. Overall, these findings indicate that poly-herbal formulations can effectively reduce ulcer severity in pyloric ligation-induced gastric injury, with Sample B emerging as the most potent candidate.

Table No. : Ulcer score and % ulcer inhibition in Pyloric Ligation induced Animals

Groups	**Poly-herbal Formulation (mg)**	**Ulcer Score**	**Ulcer Index**	**% Ulcer Inhibition**	**Gastric volume (ml)**	**Gastric pH**
Group-I	Control	0	-	-	2.84 ± 0.41	6.80 ± 0.83
Group-II	Positive control (indomethacin)	5	18.72 ± 0.28 [a]	-	8.65 ± 0.32 [a]	2.80 ± 0.21 [a]
Group-III	Negative control (Ranitidine)	1	3.17 ± 0.32 [a, b]	83.07	3.21 ± 0.23 [b, c]	6.25 ± 0.42 [b]
Group-IV	Sample in A	2	4.21 ± 0.17 [a, b, c]	63.51	4.37 ± 0.34 [a, b, c]	5.61 ± 0.72 [a, b]
Group-V	Sample in B	1	3.58 ± 0.53 [a, c]	80.88	3.31 ± 0.32 [b]	6.21 ± 0.43 [b]
Group-VI	Sample in C	3	5.42 ± 0.18 [a, b, c]	58.05	5.12 ± 0.37 [a, b, c]	3.85 ± 0.31 [a, b, c]

Values were presented as means of 6 mice ± SEM.[a, b, c] significantly different (***$p < 0.0001$) from Control group, Indomethacin (IND), and Ranitidine respectively



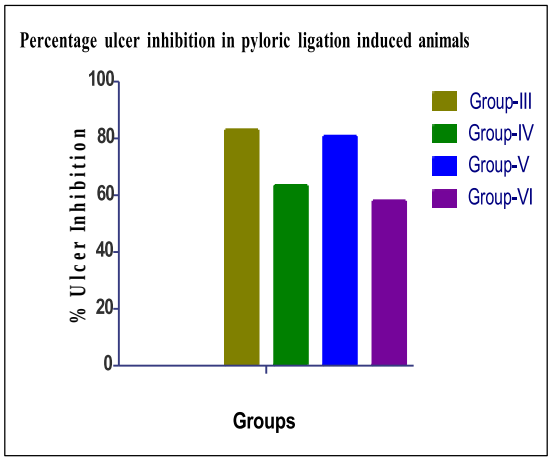
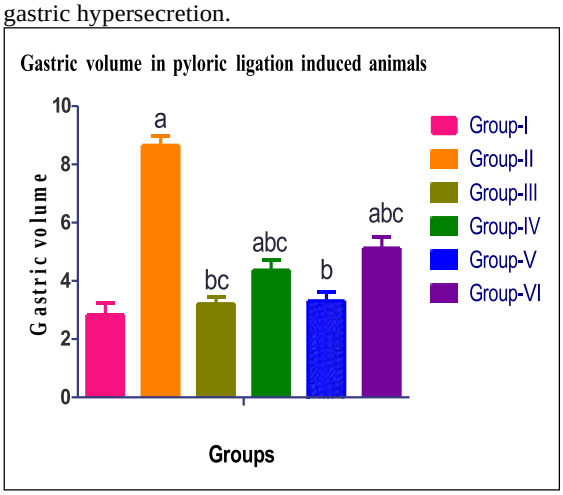


Figure : Ulcer index and % ulcer inhibition in pyloric ligation induced animals

Values were presented as means of 6 mice ± SEM.[a,b,c] significantly different (***$p < 0.0001$) from Control group, Indomethacin (IND), and Ranitidine respectively

Gastric volume and pH in Pyloric Ligation induced Animals: In the pyloric ligation model,

indomethacin administration (Group II) produced a significant increase in gastric volume (8.65 ± 0.32 mL) and a marked reduction in gastric pH (2.80 ± 0.21). Ranitidine (Group III) effectively counteracted these changes, maintaining gastric volume close to normal (3.21 ± 0.23 mL) and sustaining a near-neutral pH (6.25 ± 0.42). Among the poly-herbal formulations, Sample B (Group V) showed the most favorable results, with gastric volume (3.31 ± 0.32 mL) and pH (6.21 ± 0.43) values comparable to those of the ranitidine group. This suggests strong anti-secretory and mucosal protective activity. Sample A (Group IV) demonstrated moderate efficacy, reducing gastric volume to 4.37 ± 0.34 mL and raising pH to 5.61 ± 0.72, indicating partial protection. Sample C (Group VI) was less effective, with relatively high gastric volume (5.12 ± 0.37 mL) and lower pH (3.85 ± 0.31), reflecting weaker suppression of gastric hypersecretion.



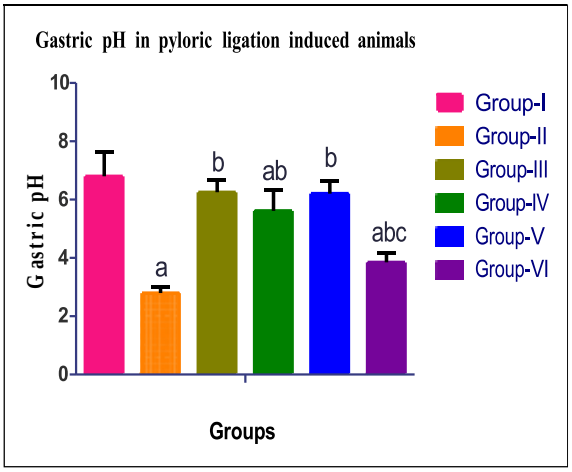


Figure : Gastric volume and pH in pyloric ligation induced animals

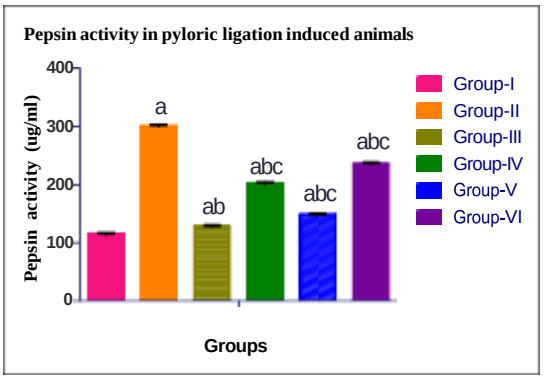
Values were presented as means of 6 mice ± SEM.[a, b, c] significantly different (***$p < 0.0001$) from Control group, Indomethacin (IND), and Ranitidine respectively

Pepsin activity and mucin content in Pyloric Ligation induced Animals: In the pyloric ligation model, indomethacin administration (Group II) produced a sharp rise in pepsin activity (301.16 ± 1.89 µg/mL). Ranitidine (Group III) maintained pepsin activity close to normal levels (128.78 ± 1.26 µg/mL) and preserved mucin content (358.82 ± 0.84 µg/mL). Among the test formulations, Sample B (Group V) demonstrated the most favorable outcome, with pepsin activity reduced to 149.27 ± 0.87 µg/mL and mucin content elevated to 337.63 ± 1.02 µg/mL. Sample A (Group IV) showed moderate efficacy, lowering pepsin activity to 201.72 ± 2.01 µg/mL and increasing mucin to 298.18 ± 1.08 µg/mL, indicating partial protection. Sample C (Group VI) was less effective, with relatively high pepsin activity (236.27 ± 1.89 µg/mL) and lower mucin levels (264.41 ± 0.91 µg/mL), reflecting weaker anti-ulcer potential.

Table No. : Pepsin activity and mucin content in Pyloric Ligation induced Animals

Groups	Poly-herbal Formulation (mg)	Pepsin activity (µg/ml)	Mucin content (µg/ml)	SOD Activity µg/mg	MDA Conc. µmol/mg
Group-I	Control	115.42 ± 1.32	375.37 ± 1.21	213.65 ± 2.73	2.56 ± 0.02
Group-II	Positive control (indomethacin)	301.16 ± 1.89 [a]	156.32 ± 0.97 [a]	102.54 ± 0.93 [a]	6.89 ± 0.06 [a]
Group-III	Negative control (Ranitidine)	128.78 ± 1.26 [a, b]	358.82 ± 0.84 [a, b]	200.73 ± 1.92 [a, b]	2.74 ± 0.02 [a, b]
Group-IV	Sample in A	201.72 ± 2.01 [a, b, c]	298.18 ± 1.08 [a, b, c]	173.06 ± 0.31 [a, b, c]	3.84 ± 0.07 [a, b, c]
Group-V	Sample in B	149.27 ± 0.87 [a, b, c]	337.63 ± 1.02 [a, b, c]	197.38 ± 0.84 [a, b, c]	2.85 ± 0.04 [a, b, c]
Group-VI	Sample in C	236.27 ± 1.89 [a, b, c]	264.41 ± 0.91 [a, b, c]	149.62 ± 0.92 [a, b, c]	5.16 ± 0.05 [a, b, c]

Values were presented as means of 6 mice ± SEM.[a,b,c] significantly different (***$p < 0.0001$) from Control group, Indomethacin (IND), and Ranitidine respectively



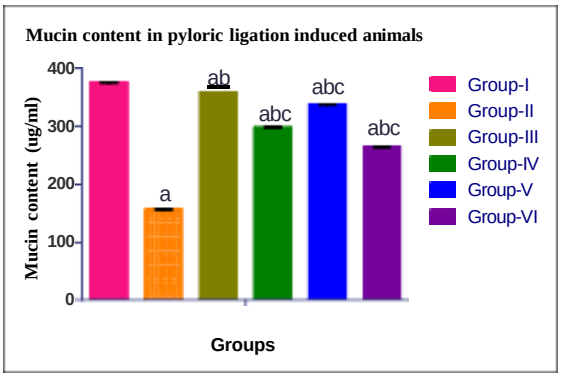
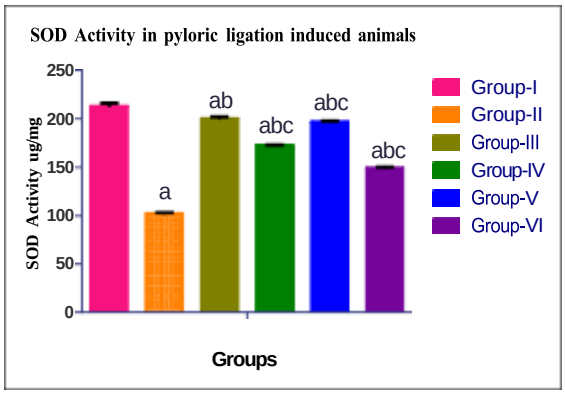


Figure : Pepsin activity and Mucin content in pyloric ligation induced animals

Values were presented as means of 6 mice ± SEM.[a,b,c] significantly different (***$p < 0.0001$) from Control group, Indomethacin (IND), and Ranitidine respectively

Assessment of stomach homogenate in Pyloric Ligation induced Animals: Indomethacin treatment (Group II) significantly reduced superoxide dismutase (SOD) activity (102.54 ± 0.93 µg/mg protein) while markedly elevating malondialdehyde (MDA) concentration (6.89 ± 0.06 µmol/mg protein). Ranitidine (Group III) preserved antioxidant status, maintaining high SOD activity (200.73 ± 1.92 µg/mg protein) and low MDA levels (2.74 ± 0.02 µmol/mg protein). Among the poly-herbal formulations, Sample B (Group V) demonstrated the strongest antioxidant potential, with SOD activity (197.38 ± 0.84 µg/mg protein) and MDA concentration (2.85 ± 0.04 µmol/mg protein) values closely approximating those of the ranitidine group. Sample A (Group IV) showed moderate efficacy, with SOD activity at 173.06 ± 0.31 µg/mg protein and MDA at 3.84 ± 0.07 µmol/mg protein, indicating partial protection. Sample C (Group VI) was less effective, with lower SOD activity (149.62 ± 0.92 µg/mg protein) and higher MDA levels (5.16 ± 0.05 µmol/mg protein), reflecting weaker antioxidant defense.



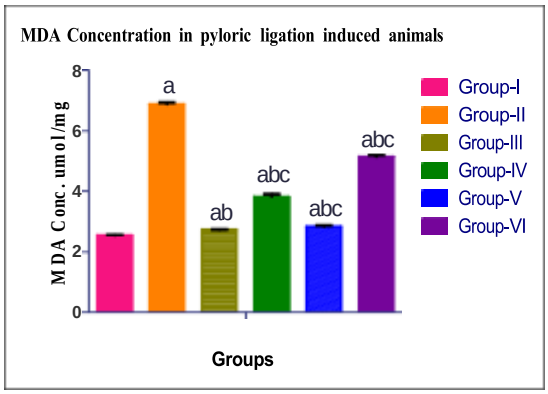


Figure : SOD Activity and MDA Conc. in pyloric ligation induced animals

Values were presented as means of 6 mice ± SEM.[a,b,c] significantly different (***$p < 0.0001$) from Control group, Indomethacin (IND), and Ranitidine respectively

Histopathology

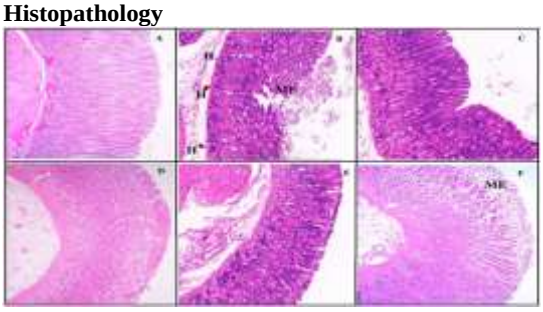


Figure : The Histopathological changes were assessed in Pyloric Ligation induced animals (A) Normal control (B) Positive control (pyloric ligation induced) (C) Negative control (Ranitidine treated) (D) Treated with sample A (E) Treated with sample B (F) Treated with sample C. Where, epithelial cell loss (EL), hemorrhage (H), inflammatory cell (leucocytes) infiltration and edema (IE) and mucosal erosions (ME)

CONCLUSION

The acute oral toxicity evaluation demonstrated that the polyherbal formulations of *Ficus benghalensis* and *Cynodon dactylon* were well tolerated at both 300 mg/kg and 2000 mg/kg doses,

with no adverse behavioral, biochemical, or necropsy findings. Consistent weight gain and stable biochemical parameters confirmed their safety under acute exposure. In ulcer models induced by indomethacin and pyloric ligation, the formulations exhibited significant gastroprotective activity, reducing ulcer scores, gastric volume, pepsin activity, and oxidative stress while enhancing mucin content and antioxidant defenses. Among the tested samples, Sample B consistently showed the highest efficacy, approaching the protective effects of ranitidine, while Sample A demonstrated moderate activity and Sample C the least. Overall, these findings highlight the safety and therapeutic promise of the polyherbal formulations, particularly Sample B, as potential candidates for the development of effective anti-ulcer agents.

CONFLICTS OF INTEREST: Nil

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