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TO INVESTIGATE THE POTENTIAL OF AERIAL ROOTS OF *FICUS BENGHALENSIS* IN INFLAMMATORY BOWEL DISEASE (IBD) IN DRUG-INDUCED RATS

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ABSTRACT

Inflammatory bowel disease (IBD) is a chronic relapsing inflammatory disorder of the gastrointestinal tract characterized by mucosal damage, oxidative stress, and excessive inflammatory responses. The present study was undertaken to investigate the therapeutic potential of the ethanolic extract of aerial roots of *Ficus benghalensis* against indomethacin-induced inflammatory bowel disease in rats. The aerial roots were subjected to Soxhlet extraction using ethanol, yielding a dark brown semi-solid extract with a percentage yield of approximately 25%. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, proteins, and terpenoids. Experimental IBD was induced by indomethacin administration, resulting in significant pathological alterations, including body weight loss, reduction in colon length, increased colon weight, elevated macroscopic lesion scores, and enhanced myeloperoxidase (MPO) activity, indicating severe intestinal inflammation. Treatment with the ethanolic extract at low and high doses significantly attenuated these disease-associated changes in a dose-dependent manner. The high-dose extract demonstrated marked improvement in body weight, restoration of colon morphology, reduction in colon edema and lesion severity, and significant suppression of MPO activity when compared with the disease control group. The therapeutic effects observed were comparable to those produced by the standard drug prednisolone. The protective activity of the extract may be attributed to the synergistic action of its bioactive phytoconstituents, particularly flavonoids, tannins, alkaloids, terpenoids, and saponins, which are known for their antioxidant, anti-inflammatory, and mucosal protective properties. The findings suggest that the aerial root extract of *Ficus benghalensis* possesses significant anti-inflammatory and intestinal protective effects and may serve as a promising natural therapeutic candidate for the management of inflammatory bowel disease. Further studies are warranted to isolate the active constituents and elucidate the underlying molecular mechanisms.

KEYWORDS: *Ficus benghalensis*, Aerial roots, Inflammatory bowel disease, Indomethacin, Myeloperoxidase, Phytochemicals, Anti-inflammatory activity.

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic, relapsing inflammatory disorder of the gastrointestinal tract that primarily comprises ulcerative colitis (UC) and Crohn's disease (CD).[1,2] These disorders are characterized by recurrent episodes of intestinal inflammation resulting from a complex interaction among genetic susceptibility, environmental factors, intestinal microbiota, and dysregulated immune responses.[3,4] IBD has emerged as a significant global health concern due to its increasing incidence, long-term morbidity, and impact on patients' quality of life.[5]

The pathogenesis of IBD involves excessive production of pro-inflammatory cytokines, oxidative stress, infiltration of neutrophils, and disruption of the intestinal epithelial barrier.[6,7] Increased levels of tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) contribute significantly to the inflammatory cascade and tissue damage observed in affected individuals.[8,9] Oxidative stress generated by reactive oxygen species (ROS) further aggravates mucosal injury, leading to ulceration, edema, and impaired healing of the intestinal lining.[10]

Current therapeutic approaches for IBD include amino salicylates, corticosteroids, immunosuppressants, and biological agents.[11,12] Although these therapies are effective in controlling disease activity, their prolonged use is often associated with adverse effects such as immunosuppression, hepatotoxicity, nephrotoxicity, and increased susceptibility to infections.[13] Furthermore, a considerable proportion of patients exhibit inadequate responses or develop resistance to existing treatments, necessitating the search for safer and more effective alternatives.[14] Medicinal plants have gained considerable attention as potential sources of novel therapeutic agents for inflammatory disorders due to their diverse phytochemical constituents and favorable safety profiles.[15] Numerous plant-derived compounds possessing antioxidant, anti-inflammatory, immunomodulatory, and wound-healing properties have demonstrated promising effects in experimental models of IBD.[16]

Ficus benghalensis Linn. (Family: Moraceae), commonly known as the banyan tree, is a large evergreen tree widely distributed throughout the Indian subcontinent.[17] Various parts of the plant, including bark, leaves, fruits, latex, and aerial roots, have been extensively utilized in traditional Ayurvedic medicine for the treatment of diarrhea, dysentery, inflammation, ulcers, diabetes, and wound healing.[18,19] Phytochemical investigations have revealed the presence of flavonoids, phenolic compounds, tannins, sterols, triterpenoids, and glycosides in the aerial roots of F. benghalensis, which are known to possess significant antioxidant and anti-inflammatory activities.[20,21] Previous pharmacological studies have reported that extracts of F. benghalensis exhibit anti-inflammatory, antioxidant, antimicrobial, antidiabetic, and gastroprotective effects.[22–24] The presence of bioactive phytoconstituents capable of scavenging free radicals and modulating inflammatory mediators suggests its potential utility in the management of inflammatory bowel disorders.[25] However, scientific evidence regarding the efficacy of aerial roots of F. benghalensis in experimental IBD remains limited.

MATERIALS AND METHODS

Selection of Plant

The choice of plant was determined based on traditional medicinal claims. Pharmacognostical research is a key factor in identifying herbal medicines derived from plants. Medicinal plants represent a significant group of economically vital species that provide essential raw materials for indigenous pharmaceutical practices. The exploration of bioactive compounds in higher plants is fundamental for discovering new medications. The detection of phytochemicals in the

ethanolic extract of *Ficus benghalensis* indicated potential antiulcerative colitis activity, leading to the selection of the aerial roots of *Ficus benghalensis* for our intended research.

Collection and Identification

Fresh aerial roots of the plant were gathered from indore, Madhya Pradesh, India. The plant was verified by Dr. S. N. Dwivedi from A.P.S. college Rewa, with the voucher specimen number J/Bot./2024-0130CSWP; . The plant material was thoroughly rinsed under tap water to eliminate dust and other impurities. Following this, the plant part was shade-dried using standard sun and air-drying methods.

Preparation of Extract

The aerial roots of *Ficus benghalensis* were washed with water to remove any physical contaminants. The cleaned roots were chopped into smaller sections and dried in the shade. After drying, they were ground into a fine powder using an electric blender. This coarsely powdered material was then stored in airtight container for subsequent analysis.

Principle

The Soxhlet extraction apparatus operates by extracting components through the condensed vapors of the solvent. When the powdered sample interacts with the condensed vapors, the soluble portions of the powder dissolve the solvent.

Extraction Procedure

The extraction process was carried out using a Soxhlet apparatus. First, the thimble was put into the Soxhlet extractor's main chamber. Next 50gm of finely ground, air-dried powder from the entire *Ficus benghalensis* aerial roots were removed and placed within the extraction chamber's thimble.[26,27] After that, 99% ethanol solvent was added to the round-bottom flask, and the extraction tube was fastened to the flask. Additionally, a reflex condenser unit was connected to the extraction tube, and water flow was permitted. The device was set up on a heating mantle, and the solvent-filled flask was heated to about 55°C. Consequently, the solvent began to evaporate and descend into the extraction tube during the condensing process. The siphon outlet was used to collect the sample. The thimble's hue was changed to clear or colorless after a 24-hour extraction operation. The same cycle was carried out again using 50gm of finely powdered, air-dried *Ficus benghalensis* aerial roots. A porcelain evaporating plate was then filled with ethanolic extract. The concentrated extract was boiled in a water bath at 55°C until it formed a semi-solid mass with a dark reddish-brown hue. The residue was used for further research after being dried, weighed, and desiccated. It was discovered that the dried residue from both extraction cycles weighed 25gms in total. The following formula was used to determine the yield %.[28]

Preliminary phytochemical screening of ethanolic extract of *Ficus benghalensis*

The extract underwent an initial phytochemical assessment to identify the presence of alkaloids, flavonoids, saponins, tannins, proteins, and terpenoids. The screening was performed following a specific protocol.

Dose selection and oral toxicity

Acute oral toxicity studies of *Ficus benghalensis* aerial root extract have demonstrated a wide margin of safety in experimental animals. Ramasamy and Kathiresan (2023) reported that administration of aerial root extract at a single

oral dose of 5000 mg/kg body weight to female Wistar rats produced no mortality, behavioural abnormalities, or significant alterations in biochemical and histopathological parameters during a 14-day observation period.

Accordingly, the oral LD_{50} of the extract was considered to be greater than 5000 mg/kg body weight. Based on the acute toxicity findings and standard pharmacological dose-selection principles, doses of 250, 500, and 1000 mg/kg body weight were selected for the present study, representing approximately 1/20th, 1/10th, and 1/5th of the maximum safe dose, respectively. These dose levels are expected to provide adequate pharmacological evaluation while maintaining a high safety margin. Agarwal, V., et al found that on aqueous aerial root extract reported an LD_{50} of approximately 2500 mg/kg body weight in rodents.[29,30]

Indomethacin-Induced Enterocolitis Model in Rats

Healthy adult male Wistar albino rats weighing between 200–250 g was selected for the study and randomly allocated into five groups, each comprising six animals (n = 5).

Experimental Animals

Healthy adult male Wistar albino rats weighing 200–250 g was procured from a registered animal facility and acclimatized to laboratory conditions for one week before the commencement of the experiment. Animals were housed under standard environmental conditions (temperature 22 ± 2°C, relative humidity 55 ± 5%, and a 12 h light/dark cycle) with free access to standard pellet diet and water ad libitum. All experimental procedures were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and were approved by the Institutional Animal Ethics Committee (IAEC).

Induction of IBD

IBD (Enterocolitis) was induced by subcutaneous administration of indomethacin at a dose of 7.5 mg/kg body weight on the 8th and 9th days of the experimental period. Indomethacin-induced intestinal inflammation is a well-established experimental model that closely mimics several pathological features of human inflammatory bowel disease, including mucosal ulceration, inflammatory cell infiltration, and intestinal tissue damage.[31]

Animal Grouping and Treatment Protocol

A total of 25 rats were randomly divided into five groups, each consisting of six animals (n = 5).

Group	Treatment	Dose and route
Group I	Normal Control	Vehicle only
Group II	Disease Control (Induced)	Indomethacin 7.5 mg/kg, s.c.
Group III	Test Extract – Low Dose + Indomethacin	Test Extract (Low Dose, p.o.) + Indomethacin 7.5 mg/kg, s.c.
Group IV	Test Extract – High Dose + Indomethacin	Test Extract (High Dose, p.o.) + Indomethacin 7.5 mg/kg, s.c.
Group V	Standard Treatment + Indomethacin	Prednisolone 2 mg/kg, p.o. + Indomethacin 7.5 mg/kg, s.c.

Dosing Schedule

The test extract and prednisolone were administered orally once daily for seven consecutive days as a pretreatment regimen. Enterocolitis was induced by indomethacin administration on Days 8 and 9. Treatment with the test extract and prednisolone was continued until Day 11. The experimental schedule is presented below.1

Day	Procedure
Day 1–7	Pretreatment with test extract or prednisolone
Day 8	Indomethacin administration (7.5 mg/kg, s.c.)
Day 9	Indomethacin administration (7.5 mg/kg, s.c.)
Day 10–11	Continuation of treatment
Day 11	Animal sacrifice and tissue collection

Tissue Collection

On Day 11, the animals were humanely euthanized by cervical dislocation under ethical guidelines. The abdominal cavity was opened immediately, and the ileum and colon were carefully excised. The test extract was administered orally once daily for seven consecutive days as a pretreatment regimen. On the 8th and 9th days, enterocolitis was induced by subcutaneous administration of indomethacin at a dose of 7.5 mg/kg. Treatment with the test extract was continued until the 11th day to evaluate its protective and therapeutic effects against indomethacin-induced intestinal inflammation. On the 11th day, all animals were euthanized by cervical dislocation under appropriate ethical guidelines, and the abdominal cavity was carefully opened for tissue collection. The ileum and colon were excised, cleaned with normal saline, and examined for the assessment of intestinal inflammation. The severity of enterocolitis was evaluated using physical and biochemical parameters, macroscopic lesion scoring, and histopathological examination of intestinal tissues. The findings obtained from the treated groups were compared with those of the disease control and standard treatment groups to determine the anti-inflammatory and protective efficacy of the test extract against indomethacin-induced enterocolitis. Macroscopic evaluation Twenty-four hours following induction of colitis, animals were euthanized by ether and 10cm of distal colon was removed from surrounding tissues, opened longitudinally along its mesenteric border, rinsed, and processed for histology. After washing the mucosa with saline solution, gross examination of mucosal injury was done using the grading scale of Morris et al.19.[32]

- Score 0 - No damage
- Score 1 - Localized hyperaemia but no ulcers
- Score 2 - Linear ulcers with no significant inflammation
- Score 3 - Linear ulcer with inflammation at one site
- Score 4 - Two or more sites of ulceration and inflammation
- Score 5 - Two or more sites of ulceration and inflammation or one major site of Inflammation and ulceration extending >1 cm along the length of the colon.

Determination of Myeloperoxidase (MPO) Activity

Myeloperoxidase (MPO) activity was estimated as a biochemical marker of neutrophil infiltration and intestinal inflammation according to the method described by Bradley et al. (1982), with slight modifications. Briefly, a 4 cm segment of inflamed colonic tissue was excised, rinsed thoroughly with ice-cold normal saline to remove fecal contents and blood, blotted dry, and accurately weighed. The tissue was minced into small pieces and homogenized in ten volumes of ice-cold potassium phosphate buffer (pH 7.4) using a tissue homogenizer. The homogenate was centrifuged at 3,500 rpm for 30 min at 4°C, and the resulting supernatant was discarded. The pellet was resuspended in 10 mL of ice-cold 50 mM potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide (HTAB) and 10 mM ethylenediaminetetraacetic acid (EDTA). The suspension was subjected to one freeze-thaw cycle followed by sonication for 15 s to facilitate the release of MPO from neutrophil granules. Subsequently, the mixture was centrifuged at 15,000 rpm for 20 min at 4°C, and the supernatant was collected for enzyme assay. After that the 0.1 mL of the supernatant was added to 2.9 mL of reaction mixture containing 50 mM phosphate buffer, 0.167 mg/mL O-

dianisidine hydrochloride, and 0.0005% hydrogen peroxide (H_2O_2). The rate of change in absorbance was measured spectrophotometrically at 460 nm using a UV–Visible spectrophotometer [33]. One unit of MPO activity was defined as the quantity of enzyme causing a change in absorbance of 1.0 per minute at room temperature. MPO activity was expressed as units per gram (U/g) of wet tissue and was calculated using the following equation:

$$\text{MPO Activity (U/g tissue)} = [100 \times (\Delta A/\text{min})] / \text{Tissue Weight (g)}$$

Were,

- ΔA/min = change in absorbance per minute at 460 nm
- Volume of supernatant = 0.1 mL
- Tissue weight = weight of colon segment used for homogenization

Increased MPO activity was considered indicative of enhanced neutrophil accumulation and severity of intestinal inflammation, whereas a reduction in MPO activity following treatment was interpreted as evidence of anti-inflammatory activity.

RESULTS

Extraction

The Soxhlet extraction method was used to generate the ethanolic extract, and its physical properties are displayed in (table no. 6)

Percentage Extractive Value and Physical Characteristic of Extract				
Extract	**% Dry wt. (w/w)**	**Colour**	**Odor**	**Consistency**
Ethanolic extract	25%	Dark brown	characteristic	Sticky (semi solid)

7.2% yield of extracted compound

The percentage yield was calculated. Using the formula below and computing the yield extract percentage, the percentage of extracted chemical was determined to be 25%. The formulation is made using the extract.

- % yield = Practical yield/theoretical yield × 100
- % yield = 25/100 × 100
- % yield = 25%

Phytochemical screening test

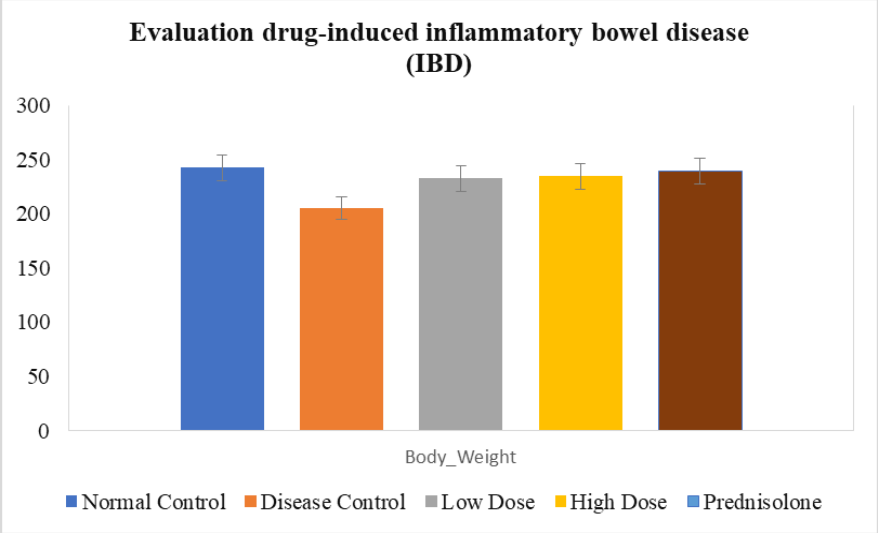
Table No. 5: Preliminary phytochemical analysis

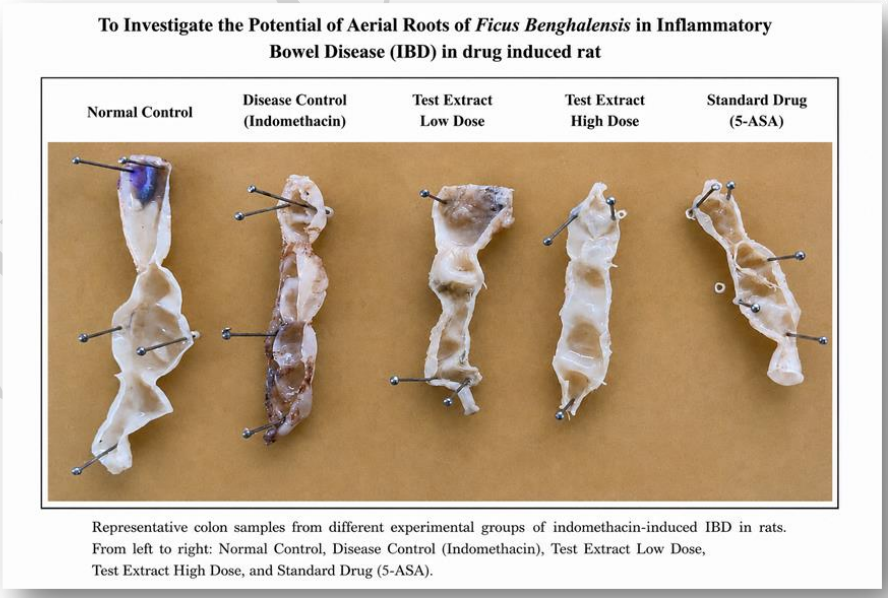
S. No.	Constituents	Presence/absence
1	Alkaloids	positive
2	Flavonoids	positive
3	Saponins	positive
4	Tannins	positive
5	Proteins	positive
6	Terpenoids	positive

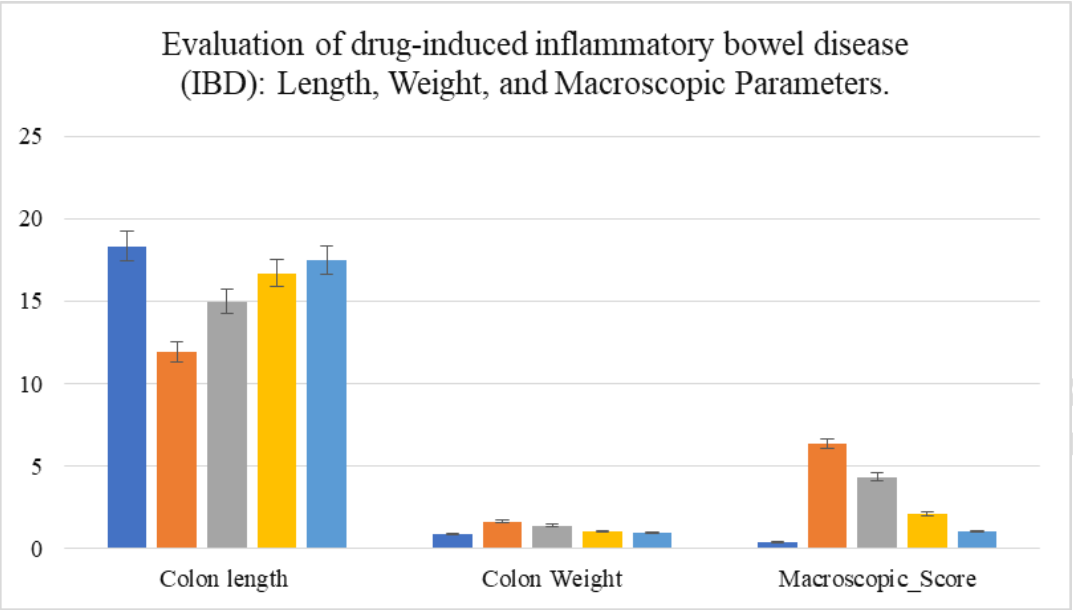
Alkaloids, flavonoids, saponins, tannins, protein, terpenoids, and other compounds were detected by preliminary phytochemical analysis of the extract. The protocol was followed when conducting the phytochemical screening. (O.P. Ogunlowo et al.,2013)

Evaluation of the protective and therapeutic effects in drug-induced inflammatory bowel disease (IBD)				
Group	**Body weight**	**Colon length**	**Colon Weight**	**Macroscopic Score**
Normal Control	242.56±3.21	18.32 ±0.13	0.84 ±0.26	0.39± 0.04
Disease Control	205.04±2.8	11.91 ±0.22***	1.61±0.25**	4.98 ±.0.23***
Low Dose	232.46±2.06	14.98 ±0.15*	1.39±0.35*	4.11±.021*
High Dose	234.54± 3.1*	16.7 ±0.21**	1.04±0.33*	2.09±1.03*
Prednisolone	239.46±3.44*	17.46 ±0.19***	0.95±0.41**	1.0± 0.85**

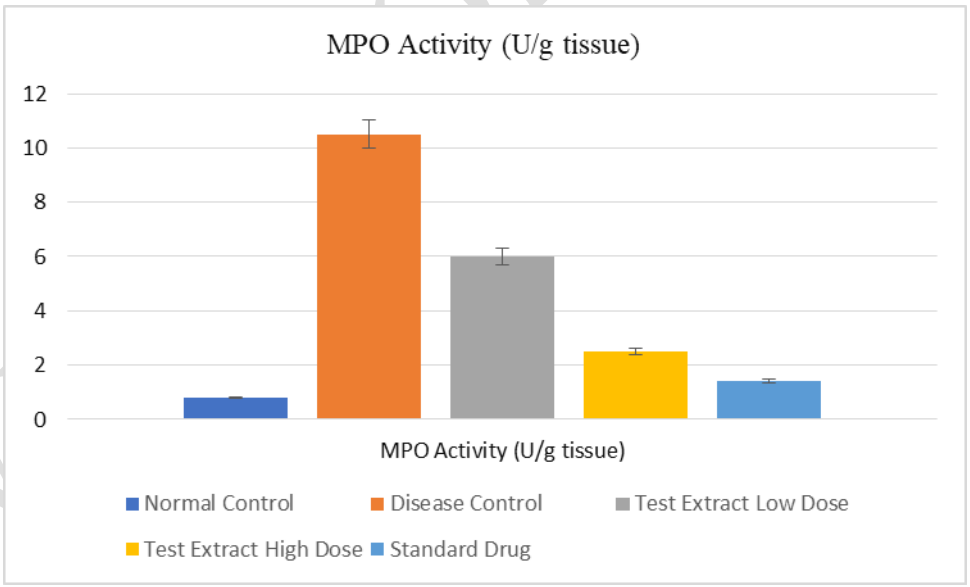
Data are expressed as Mean ± SEM (n = 5). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. A statistically significant difference was observed among the experimental groups for body weight, colon length, colon weight, and macroscopic score, activity. Disease control animals exhibited significant deterioration compared with the normal control group ($p < 0.001$). Treatment with the test extract produced dose-dependent protection against indomethacin-induced intestinal inflammation. The high-dose extract significantly improved body weight, colon length, colon weight, and macroscopic lesion score activity compared with the disease control group ($p < 0.01$ to $p < 0.001$). The effects of the high-dose extract were comparable to those observed with prednisolone treatment.







Group	MPO Activity (U/g tissue)
Normal Control	0.8 ± 0.1
Disease Control	10.5 ± 0.4****a
Test Extract Low Dose	6.0 ± 0.3**b
Test Extract High Dose	2.5 ± 0.2**b, c
Standard Drug	1.4 ± 0.2**b
Based on the reported MPO activity values (Mean ± SEM), a One-Way ANOVA followed by Tukey's Multiple Comparison Test can be used to compare all groups, **a** P < 0.0001 vs Normal Control P P < 0.01vs Disease Control P < 0.01 vs Standard Drug	



DISCUSSION

The present study investigated the protective and therapeutic potential of the ethanolic extract against indomethacin-induced inflammatory bowel disease (IBD) in experimental animals. Soxhlet extraction yielded a dark brown, semi-solid extract with a percentage yield of 25%, indicating efficient extraction of bioactive constituents. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, tannins, proteins, and terpenoids.

These phytoconstituents are well documented for their antioxidant, anti-inflammatory, and mucosal protective properties, suggesting their possible contribution to the observed pharmacological effects.

Indomethacin administration produced characteristic manifestations of intestinal inflammation, as evidenced by a significant reduction in body weight and colon length, along with increased colon weight and macroscopic lesion scores in the disease control group. These findings are consistent with previous reports demonstrating that non-steroidal anti-inflammatory drugs induce severe intestinal damage through oxidative stress, inflammatory mediator release, and disruption of mucosal integrity. The marked increase in colon weight and macroscopic score further reflects edema formation, inflammatory cell infiltration, and tissue injury associated with experimental IBD. Treatment with the ethanolic extract resulted in significant amelioration of these pathological changes in a dose-dependent manner.

Animals receiving the low-dose extract showed moderate improvement in body weight, colon morphology, and lesion scores, whereas the high-dose extract produced more pronounced protection. Restoration of colon length and reduction in colon weight indicate attenuation of intestinal inflammation and edema. Furthermore, the reduction in macroscopic damage scores suggests preservation of mucosal architecture and decreased severity of intestinal lesions. The therapeutic efficacy of the high-dose extract approached that of prednisolone, a standard anti-inflammatory drug, highlighting its potential as a promising natural therapeutic agent for IBD management.

Myeloperoxidase (MPO) activity was assessed as a biochemical marker of neutrophil infiltration and intestinal inflammation. The disease control group exhibited a marked elevation in MPO activity compared with the normal control group ($P < 0.0001$), confirming extensive inflammatory cell recruitment to the intestinal tissue. Administration of the ethanolic extract significantly reduced MPO activity in a dose-dependent manner. The high-dose extract produced a substantial decrease in MPO levels, approaching values observed in the standard drug-treated group. This reduction indicates effective suppression of neutrophil accumulation and inflammatory responses within the intestinal mucosa. The observed decrease in MPO activity supports the macroscopic findings and further confirms the anti-inflammatory activity of the extract. The beneficial effects observed in this study may be attributed to the synergistic action of the phytoconstituents present in the extract. Flavonoids and tannins are known to scavenge reactive oxygen species and inhibit pro-inflammatory cytokine production, while alkaloids and terpenoids possess immunomodulatory and anti-inflammatory activities. Saponins have also been reported to enhance mucosal healing and reduce inflammatory damage. Therefore, the combined action of these bioactive compounds may account for the significant protection against indomethacin-induced intestinal injury.

Overall, the findings demonstrate that the ethanolic extract possesses significant anti-inflammatory and intestinal protective effects in experimental IBD. The extract improved clinical, morphological, and biochemical parameters associated with intestinal inflammation, with the high dose showing efficacy comparable to prednisolone. These results support the potential therapeutic value of the extract in the management of inflammatory bowel disease and warrant further investigation to identify the active constituents and elucidate the underlying molecular mechanisms.

CONCLUSION

The present investigation demonstrated that the ethanolic extract of aerial roots of *Ficus benghalensis* exhibits significant protective and therapeutic effects against indomethacin-induced inflammatory bowel disease in rats.

Phytochemical analysis confirmed the presence of several bioactive constituents, including alkaloids, flavonoids, tannins, saponins, and terpenoids, which are known to possess potent antioxidant and anti-inflammatory activities.

Administration of indomethacin produced characteristic features of intestinal inflammation, including reduction in body weight and colon length, increased colon weight, elevated macroscopic lesion scores, and increased MPO activity.

Treatment with the aerial root extract significantly reversed these pathological alterations in a dose-dependent manner.

The high-dose extract produced substantial improvement in clinical, morphological, and biochemical parameters and showed efficacy comparable to the standard drug prednisolone. The reduction in MPO activity indicates suppression of neutrophil infiltration and inflammatory responses within the intestinal mucosa, while improvements in colon morphology suggest protection against mucosal damage and edema. These findings support the role of *Ficus benghalensis* aerial roots as a potential source of natural anti-inflammatory agents for the treatment of IBD.

In conclusion, the ethanolic extract of aerial roots of *Ficus benghalensis* possesses promising anti-inflammatory and intestinal protective properties and may represent a valuable phytotherapeutic approach for inflammatory bowel disease. However, further pharmacological, toxicological, and molecular studies are required to identify the active principles, establish long-term safety, and clarify the mechanisms responsible for its therapeutic efficacy. Based on the present findings, *Ficus benghalensis* aerial roots hold considerable potential for future development as a novel herbal intervention in IBD management.

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