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In-vivo Pharmacological Evaluation of Anti-Ulcerative Colitis Activity of Ethanolic Bark Extract of *Ficus religiosa* in Swiss Albino Mice

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Abstract: Ulcerative colitis is an idiopathic chronic disease of the intestine with unknown etiology involving multiple immune, genetic and environmental factors. The present study was carried out based on traditional claims to assess anti-ulcerative colitis activity. The extraction of the bark powdered of *Ficus religiosa* was carried out using ethanol as a solvent in the soxhlet apparatus. The % yield of the ethanol extract of *Ficus religiosa* was calculated and it was found to be 21%. The quantitative preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, saponins, amino acids, and protein in ethanolic extract of *Ficus religiosa*. Ulcerative colitiswas induced in mice by induction of 2ml of 3% acetic acid through intrarectally. The low dose and high dose (200mg/kg and 400mg/kg) of extract was administered orally in mice. Prednisolone (1.14mg/kg) was used as the standard drug for comparison. All acetic acid- induced mice showed typical clinical manifestations of ulcerative colitis. Colon length increased significantly and colon weight decreased significantly of the treatment groups when compared to the standard groups. The MPO activity is significantly higher in *Ficus religiosa* treated group (200mg/kg and 400mg/kg) in comparison to standard drug Prednisolone (1.14mg/kg) treated group. It was observed that the inflammation in the colonic tissue of *Ficus religiosa* treatment group was significantly higher than standard group. The two treatment group shows nearly equal effect as that of the standard drug. All parameters suggest that the 400 mg/kg dose is more effective than the lower dose.
Keywords: Ulcerative colitis, Ficus religiosa, Prednisolone, soxhlet apparatus.

Introduction

Ulcerative colitis (UC) is a chronic inflammatory bowel disease with an unknown etiology, but some of the factors affecting both the initiation and progression of the disease such as immune, genetic, and environmental factors. Oxidative stress is a key factor in pathogenesis and perpetuation of the mucosal damage in ulcerative colitis. Neutrophils and monocytes were responsible to produce high concentrations of oxygen-derived free radicals in ulcerative colitis patients. The ultimate goal for ulcerative colitis treatment is complete remission; thus, a phytonutrient-rich plant with reported antioxidant and anti- inflammatory properties would be

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beneficial to ulcerative colitis treatment remains a progressive study that requires continual optimization.

The reported phytoconstituents of stem bark of *F. religiosa* are phenols, tannins, steroids, alkaloids and flavonoids, β-sitosteryld-glucoside, vitamin K, n-octacosanol, methyl oleanolate, lanosterol, stigmasterol, lupen-3-one. It has been reported that the aqueous extract of the dried bark of *F. religiosa* contains tannins, phytosterols, flavonoids, and derivatives of furanocoumarins, specifically begaptol and bergapten these phtoconstituents facilitate anti-inflammatory action. The reported medicinal properties of Ficus religiosa areantimicrobial, anti-daibetic, antihyperlipidaemic activity, wound healing, Anticonvulsant activity, Antibacterial activity. This study was undertaken to determine the anti-ulcerative colitis effect of *Ficus religiosa* extract in the acetic acid-induced ulcerative colitis in mice.

MATERIAL AND METHODS

Chemicals: Acetic acid, Ethanol, and Prednisolone standard drug.

Collection & Authentification of plant material: The fresh bark of *Ficus religiosa* was collected from Mhow area, Indore, Madhya Pradesh, India. The plant was authenticated by Dr. S.N. Dwivedi, Department of Botany, Janata PG College, A.P.S. University, Rewa, Madhya Pradesh, India as *Ficus religiosa* (family-Moraceae) in the provided Voucher Specimen Number: J/Bot./2023-0134CSWP.

Preparation of extracts

The stem bark were cleaned with the running tap water, air dried and ground into coarse powder. The powder (75 g) was extracted with ethanol using the soxhlet apparatus and water bath. The yield extract of the *Ficus religiosa* is 21%. The obtained thick brown semi solid extract was stored at 2–4 ℃ for further use. [4]

Experimental Animals:

Healthy Swiss albino mice weighing 20-25g of either sex was obtained from the animal house of Swami Vivekanand college of pharmacy, Indore for the evaluation of anti-

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ulcerative colitis activity. The Institutional Animal Ethical Committee of our organization gave its approval to the experimental protocol. (Approval No: IAEC/SVCP/2023/02) and conformed according to CPCSEA regulations. Committee for the Purpose of Control & Supervision of Experiments on Animals, India (also referred to CPCSEA) New Delhi.

Acute oral Toxicity:
According to literature ethanolic extract of bark of *Ficus religiosa* was found safe at 400 mg/kg and 2g/kg body weight, no mortality was observed at both doses and therefore, LD50 of ethanolic extract of plant was found to be 2000 mg/kg body weight.(5)

Experimental design:
Healthy swiss albino mice of either sex were used for the study. Twenty mice were divided into five experimental groups as follows:

S.No.	Groups	Number of animals
1.	Normal Control	2
2.	Negative Control	3
3.	Low dose of test extract (200 mg/kg)	5
4.	High dose of test extract (400 mg/kg)	5
5.	Standard Drug prednisolone (1.14 mg/kg)	5
6.	**Total**	**20**

Table no. 1 Grouping of mice.

Procedure: The mice were weighed and marked individually. Group 1 (Normal control) mice were treated with the normal saline for 6 consecutive days. Group 2 (Negative control) mice were only treated with 2ml of 3% acetic acid to induce colitis. Group 3 (Low dose of test extract) experimental mice were treated with low dose of bark extract *Ficus religiosa* (200mg/kg/bwt) and group 4 (High dose of test extract) was treated with high dose of bark extract of *Ficus religiosa* (400 mg/kg/bwt). Based on the results of earlier in vivo drug toxicity studies, the treatment dose that was used in this investigation was selected. Group 5 (Standard dose) was treated with standard drug prednisolone (1.14 mg/kg/bwt). All these drugs were given orally for 6 consecutive days. After one day fasting, except group 1 mice all other experimental

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group mice were induced for ulcerative colitis with single dose of 2ml of 3% acetic acid and volume given was according to the body weight of mice and introduced by using polyethylene tube of 2 mm in diameter through the rectum into the colon region n up to 8 cm. The mice were kept in supine Trendelenburg position for 30s to hinder the intracolonic instillate leakage. The mice were sacrificed on 7th day and the colon tissues were dissected out for analysis. The dissected colonic specimens were washed with ice cold PBS (pH 7.2) and stored in 10% formalin for the histopathology and for morphologic studies**.** [1,9]

Evaluation of ulcerative colitis:

a. Evaluation of colon length and colon weight:

- **Determination of Colon length:** 10 cm distal portion of each mice colon was removed after sacrifice. The colon was dissected longitudinally and washed with normal saline to remove fecal materials. Inflammation was scored macroscopically based on clinical features of the colon using the scale ranging from 0 to 4 as follows: 0 (no macroscopic changes), 1 (only mucosal erythema), 2 (mild mucosal edema) 3 (moderate edema), and 4 (severe ulceration)**.**
- **Determination of Colon weight:**
After sacrifice a 5-cm segment of distal colon, 3 cm proximal to anus was resected to determine the weight of the colon. Weight of the colon were measured and expressed in milligrams per centimeter (mg/cm) and the mean ± SD for every group was calculated.

b. Evaluation of MPO activity:

MPO activity was measured according to a previously described method. Briefly, colon tissue (100 mg) was homogenized in 1 ml of phosphate buffered saline (PBS; pH 7.0) containing hexadecyltrimethylammonium bromide (HTAB; 0.5%) and ethylenediaminetetraacetic acid (5 mm, pH 7.4). After centrifugation at 4000 rpm for 15 min at 4°C, the supernatant was assayed in reaction medium consisting of 50 mm PBS (pH= 7.4) containing 0.167 mg/mL o-dianisidine dihydrochloride and 0.005% H2O2. MPO activity was measured using a spectrophotometer at 460 nm. One unit of MPO activity was defined as the amount of MPO required to degrade 1 µM hydrogen peroxide (H_2O_2) per minute at 25°C. The results of the MPO assay are expressed as units per milligram (U/mg) of protein. This activity was performed by pathologist in pathology.

c. Serological evaluation of C Reactive Protein (CRP):

Mice were anaesthetized and blood samples were collected from retro-orbital plexus. Serum was separated from whole blood by centrifugation at 3000 rpm and stored at -20℃ for CRP test which was performed by the pathologist. Individual with ulcerative colitis showed increased level of CRP.

d. Histopathological study:

The histopathological analysis was done in the colon specimen fixed in 10% formalin in PBS and embedded in paraffin. About 4mm thick sections of colon were prepared, stained with Eosin

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and Hematoxylin and observed under light microscope. All sections were analyzed and interpreted by a certified histopathologist.

4. **Statistical analysis:**

Mean ±SD was used for all data and value expression. All other experimental data were analyzed using one- way ANOVA followed by Tukey's multiple comparisons test. Graphs were plotted using GraphPad Prism for Windows version 8.01 (GraphPad).

5. **Results:**

The % yield of ethanolic extract of bark of *Ficus religiosa* was 21%.

Qualitative phytochemical analysis of *Ficus religiosa* extract:

The qualitative phytochemical analysis indicated that the ethanolic extract of bark of *Ficus religiosa* contains alkaloids, steroids, tannins, saponins, amino acid & proteins and flavoniods.

Assessment of ulcerative colitis activity:

a. Effect of *Ficus religiosa* extract on colon length and colon weight: Acetic acid induced colitis is associated with marked decrease in colon length and increased in colon weight. As evidence table no. 2 colon length was decreased significantly and colon weight was slightly increased as compared to the normal control. In low dose and high dose treatment (200mg/kg and 400mg/kg) group, length of low dose of test extract group is slightly decreased and high dose of test extract group is increased when compared to standard group. Significance of colon length is $p<0.012$. A significant increase in colon weight was observed in negative control group when compared with normal group. In low dose and high dose treatment (200mg/kg and 400mg/kg) group due to the treatment of *Ficus religiosa* bark extract the colon weight significantly increased (**$p<0.02$) as observed in standard drug treatment group. In comparison of low dose and high dose treatment (200mg/kg and 400mg/kg) group the colon weight is significantly decreased in high dose of test extract group.

Groups	**Colon length (cm)**	**Colon weight (mg)**
Normal Control	7.55±0.035	289.64±0.18
Negative control	5.74±0.005	362.55±0.25
Low dose of test extract (200mg/kg)	5.95±0.035	352.62±0.12
High dose of test extract (400mg/kg)	6.52±0.05	319.74±0.56
Standard drug (Prednisolone)	6.58±0.045	305.67±0.32

Table no. 2 Assessment of colon length and colon weight.

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Data are expressed in Mean ± SEM and results were analyzed by ANOVA using Tukey's multiple comparison test: Significance of colon length at $p<0.012$

Data are expressed in Mean ± SEM and results were analyzed by ANOVA using Tukey's multiple comparison test: Significance of colon weight at $^*p<0.02$.

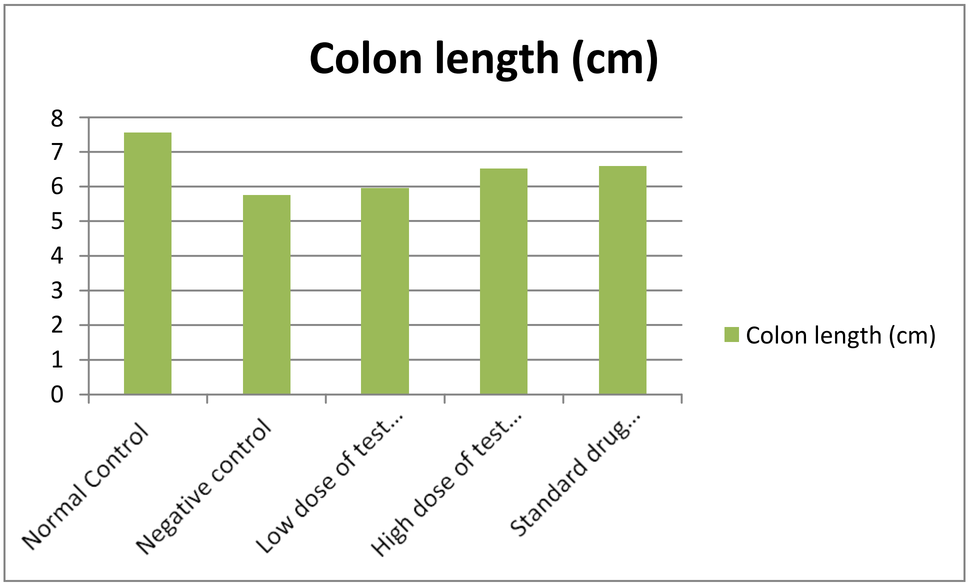

Fig.1 Colon length of different experimental mice.

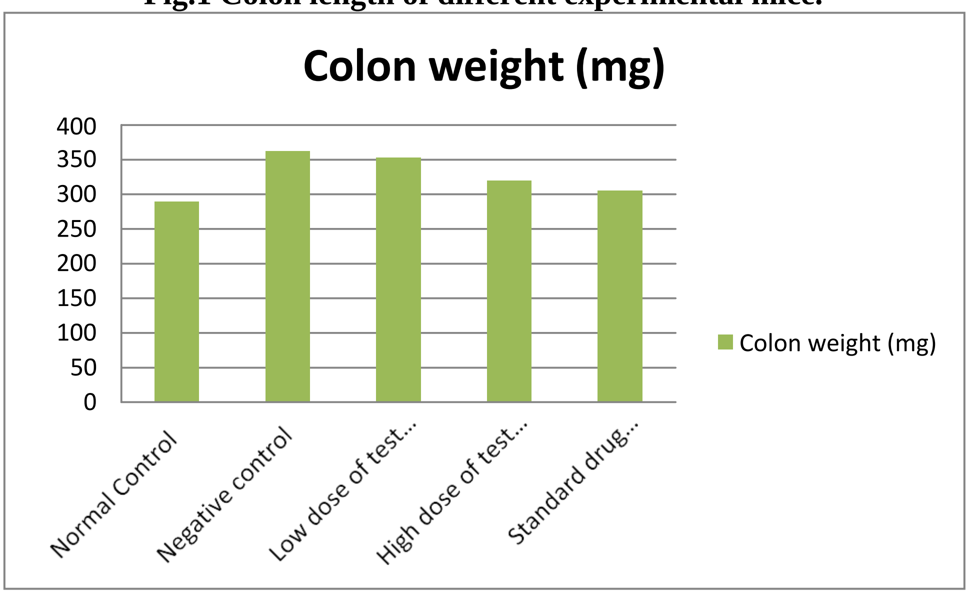

Fig.2 Colon weight of different experimental mice.

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b. Myeloperoxidase Activity (MPO) Changes: The changes in the activity of MPO in ulcerative colitis homogenates of treated animals is shown in table no. 3. The MPO activity of negative control group is (142) showed significantly increase in comparison to normal control (50). The MPO activity decreased in *Ficus religiosa* treated group (200mg/kg and 400mg/kg) and standard drug (Prednisolone) treated group (1.14mg/kg) in comparison to normal control group.

S.No.	Groups	Mean±SEM
1.	Normal Control	50.5±0.5
2.	Negative control	142±4.5
3.	Low dose of test extract (200mg/kg)	64±2.0
4.	High dose of test extract (400mg/kg)	59.2±2.5
5.	Standard drug Prednisolone (1.14mg/kg)	55±3.0

Table No. 3 Assessment of Myeloperoxidase (MPO) activity.

Note: Data are expressed as mean ± SEM and results were analyzed by ANOVA using Tukey's multiple comparison test: Significance at* $p<0.01$ Vs control.

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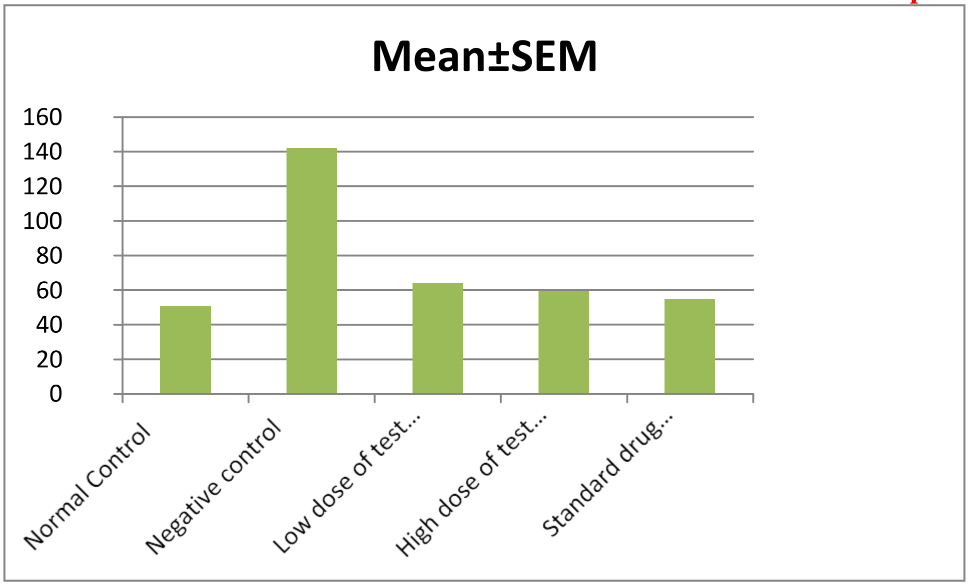

Fig.3 MPO Level (ng/ml) of different experimental mice.

C. Serological evaluation i;e. C-Reactive Protein (CRP):

The colonic tissue of the negative control (0.47mg/dl) has more inflamed in comparison to the normal control (0.25mg/dl). It was observed that the inflammation in the colonic tissue of *Ficus religiosa* treatment group was significantly higher than standard group.

S.No.	Groups	Mean±SEM
1.	Normal control	0.25±0.00
2.	Negative control	0.47±4.5
3.	Low dose of test extract (200mg/kg)	0.39±0.025
4.	High dose of test extract (400mg/kg)	0.33±0.012
5.	Standard drug Prednisolone	0.30±0.035

Table No. 4 Assessment of C-reactive protein (CRP).

Note: Data are expressed as mean ± SEM and results were analyzed by ANOVA using Tukey's multiple comparison test: Significance at* p value is 0.02772 Vs control**.**

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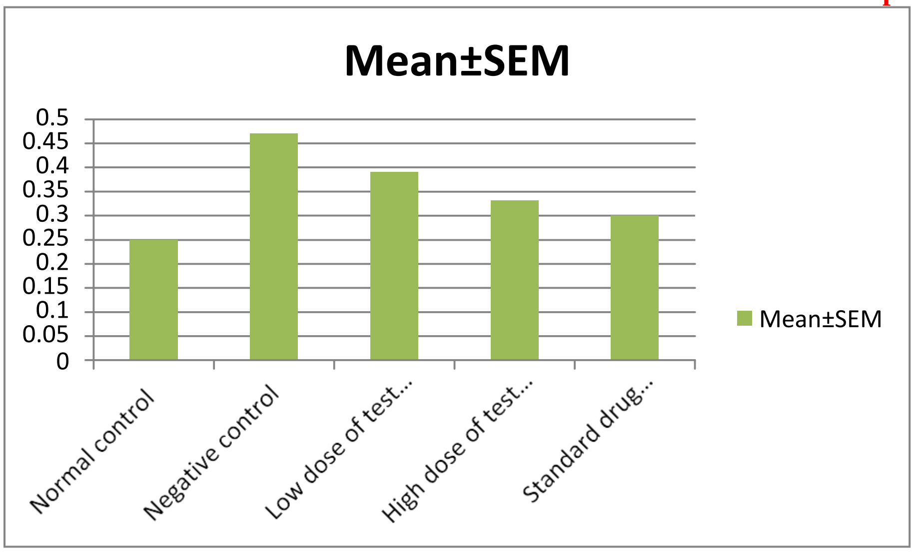

Fig.4 CRP level (mg/dl) of different experimental mice.

D. Histopathology:

As a result of microscopic examinations, scores of colon tissues of mice in negative controlgroup was significantly higher in comparison of normal control group. However, thehistopathological scores were observed improved by treatment with (200mg/kg and 400mg/kg) and standard drug when compared to the negative control group.

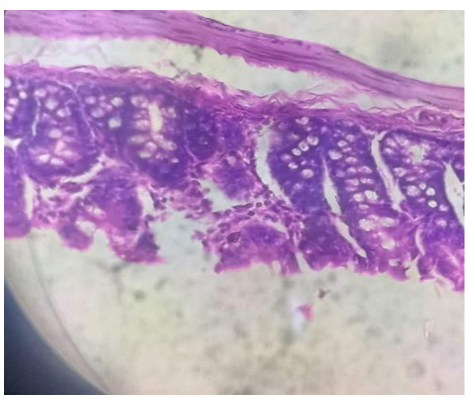

Fig. 5 Histopathological image of Normal Control group.

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Fig.6 Histopathological changes on administration of acetic acid in negative controlgroup.

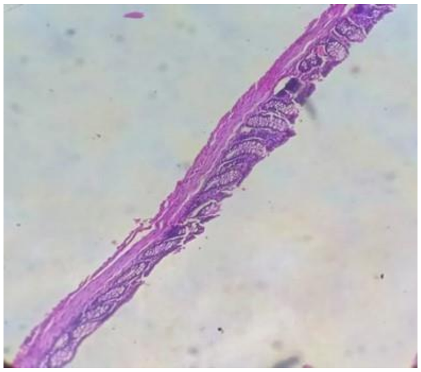

Fig.7 Histopathological changes on administration of low dose of test extract(200mg/kg)

Fig.8 Histopathological changes on administration of high dose of test extract(400mg/kg)

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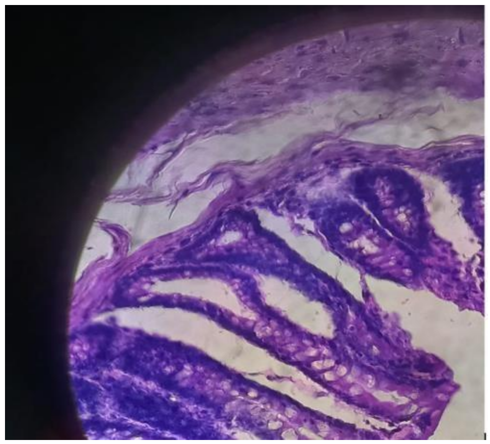

Fig. 9 Histopathological changes on administration of standard drug (Prednisolone)

6. Discussion:

This study investigates the anti-ulcerative colitis activity of Ficus religiosa bark extract. Utilizing ethanol extraction, a 21% yield was obtained. Phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, saponins, amino acids, and proteins. Acetic acid-induced ulcerative colitis in mice was treated with two doses of Ficus religiosa extract (200mg/kg and 400mg/kg), comparing favorably with prednisolone. Results showed a significant decrease in colon length, increased in colon weight. The MPO activity decreased in *Ficus religiosa* treated group (200mg/kg and 400mg/kg) and standard drug (Prednisolone) treated group (1.14mg/kg) in comparison to normal control group. In CRP activity the colonic tissue of the negative control (0.47mg/dl) has more inflamed in comparison to the normal control (0.25mg/dl). It was observed that the inflammation in the colonic tissue of *Ficus religiosa* treatment group was significantly higher than standard group. It was observed that the higher dose (400mg/kg) exhibited superior efficacy.

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7. Conclusion:

The present experimental protocol showed that the bark of *Ficus religiosa* elicited a significant anti-ulcerative colitis activity by acetic acid model. The anti-ulcerative colitis activity of ethanolic extract is comparable with prednisolone, hence it is useful in treatment of ulcerative colitis activity. The anti-ulcerative colitis possessed by *Ficus religiosa* ethanolic bark extract is being reported for the first time. The phytochemical constituents of the plant revealed the presence of alkaloids, flavonoids, saponin, cardiac glycosides, tannins and amino acids &protein. The observed pharmacological activities might have been attributed to the presence of active phytochemical constituents in the ethanolic extract. The results of this investigation indicate that the ethanolic bark extract of *Ficus religiosa* 200 mg/kg and 400 mg/kg has marked as increased in colon length and colon weight as compared to control group. However, further pharmacological investigation using isolated active ingredients can be carried out to confirm its efficacy and mechanism of action.

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