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Anti-Ulcerogenic Potential of *Ficus Bengalensis* Linn. Bark in Experimental Rats

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ABSTRACT

The present study was performed to evaluate the anti-ulcerogenic activity of hydro-alcoholic extract of *Ficus bengalensis* Linn. bark against indomethacin-induced ulcers in rats and swimming stress induced ulcers in rats. Five groups of adult wistar rats were orally pre-treated respectively with carboxy methyl cellulose (CMC) solution (ulcer control group), Omeprazole 20 mg/kg (reference group), and 100, 200 and 300 mg/kg *F. bengalensis* Linn. bark extract in CMC solution (experimental groups), one hour before oral administration of indomethacin to generate gastric mucosal injury. Rats were sacrificed and the ulcer index, gastric volume, gastric pH, free acidity, total acidity of the gastric content was determined. Grossly, the ulcer control group exhibited severe mucosal injury, whereas pre-treatment with *F. bengalensis* Linn. bark extract exhibited significant protection of gastric mucosal injury in both models. Histological studies revealed that ulcer control group exhibited severe damage of gastric mucosa, along with edema and leucocytes infiltration of submucosal layer compared to rats pre-treated with *F. bengalensis* Linn. bark extract which showed gastric mucosal protection, reduction or absence of edema and leucocytes infiltration of submucosal layer. Acute toxicity study did not manifest any toxicological signs in rats. The present finding suggests that *F. bengalensis* Linn. bark extract promotes ulcer protection as ascertained grossly and histologically compared to the ulcer control group.

Key Words: *F. bengalensis* Linn., Hydro-alcoholic, Gastric ulcer.

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INTRODUCTION

Peptic ulcer is one of the most prevalent gastrointestinal disorders, commonly occurs in developed countries. Treatments available for ulcer is generally non-specific and is usually aimed at reducing the production of gastric acid and re-enforcing gastric mucosal protection such as regular food, adequate rest and avoidance of ulcerogenic agents such as coffee, alcohol and tobacco. The drugs used in the treatment of ulcer include receptor blockers, proton pump inhibitors, drugs affecting the mucosal barrier and act on the central nervous system. Even though a range of drugs are available for the treatment of ulcer, many of these do not fulfill all the requirements and have side effects[1].

Ficus bengalensis (Moraceae) is commonly known as a Banyan tree or Vata or Vada tree in ayurveda. The tree sends down its branches and great number of shoots, which take root and become new trunks. The plant is a large evergreen tree distributed all over India from sub Himalayan region and in the deciduous forest of Deccan and south India. It is a grown in gardens and road sides for shades[2].

The uses reported are anti-inflammatory[3], antioxidant[4], antidiarrhoeal[5], antidiabetic activity[6] etc. Many plants of this genus are used in medicine for the treatment of skin diseases, enlargement of liver and spleen, dysentery, diarrhoea, diabetes, leprosy, lung complaints, leucorrhoea, heart diseases, cough, asthama, piles, ulcers, gonorrhoea, rheumatism and lumbago[7].

In the present study we have evaluated the anti-ulcer (ulcer preventive) activity and acute toxicological effects of *F. bengalensis* Linn. hydro-alcoholic extract of bark in the present investigation.

MATERIALS AND METHOD

Chemicals and Reference Drug:

All chemicals used in the present study were analytical grade and purchased from Merck specialties Pvt. Ltd. (Mumbai, India). Omeprazole (reference drug) from Dr. Reddy's laboratories (Hyderabad, India).

Plant Material:

The bark *Ficus bengalensis* Linn. were collected from local areas of Pune, Maharashtra and authenticated by Dr. P. G. Diwakar, Joint Director, at Botanical Survey of India (BSI), Govt. of India, Ministry of Environment and Forests, Pune, India.

Preparation of Extract:

The air-dried bark of *Ficus bengalensis* Linn. powdered (40 size mesh) and around 500 gm of powder was subjected to extraction (soxhlet) with petroleum ether to defatt the powder. Each time before extracting with next solvent the powdered material was dried at room temperature. After the effective extraction, solvent were concentrated using rotary evaporator and water was removed. The obtained extracts were subjected to chemical investigation and pharmacological screening for its anti-ulcer activity.

Phytochemical Screening:

The freshly prepared crude Hydro-alcoholic extract of *Ficus bengalensis* Linn. Bark was qualitatively tested for the presence of major phytochemical constituents. This was carried out by the method described by J. B. Harborne, 1984.[8]

Animals:

Wistar rats (180-200 g) and male albino mice (20-25 g) were obtained from the animal Centre, Pune and kept in standard environmental conditions. They were fed with standard pellet diet and water *ad libitum*. Experiments were carried out in accordance with CPCSEA guidelines and the study was approved by Institutional animal ethical committee.

Acute Toxicity Study:

Five groups (n=6) of male albino mice were used in the acute toxicity study of *Ficus bengalensis* Linn. Hydro-alcoholic extract. Animals from all groups were fasted overnight and administered (p.o) with single dose (250, 500, 2000 and 5000 mg kg^{-1}) of the extract. A group of animals which received equal volume of 1% CMC served as control. Acute toxicity study was perform as per 423 guidelines (OECD)[9]. Changes in the behavior of animals were observed for 24 h after extract administration. For any signs of toxicity and mortality, animals were observed for 14 days.

Indomethacin Induced Ulcers In Rats:

The albino rats of either sex weighing between 180-200 gm were divided into five groups of six animals each and fasted for 24 hrs with water *ad libitum* prior to experiment. The animals of group I were pretreated with vehicle (1% Sodium CMC aqueous solution) 1.0 ml/kg p.o. and the animals of group II were treated with standard i.e. Omeprazole (20 mg/kg p.o.). Similarly animals of group III, IV and V were pre-treated with hydro-alcoholic extract 100mg/kg, 200mg/kg, 300mg/kg p. o. respectively. Indomethacin (30mg/kg p.o.) was administered to the animals of all groups, 60 minutes after the respective treatments. 4 hrs later, the animals were sacrificed by cervical dislocation, and the stomachs were removed and opened along the greater curvature and ulceration

was scored. The percentage protection was calculated. The number of ulcers per stomach was noted and severity of the ulcers was observed microscopically and scoring was done as follows:

0 - Normal Mucosa

0.5 - Red colouration

1.0 - Spot ulcers

1.5 - Haemorrhagic streaks

2.0 - Ulcers >3 but <5

2.5 - Ulcer >5

Mean ulcer score for each animal is expressed as ulcer index.

$$\% \text{ Protection} = \frac{(\text{UI control} - \text{UI treated}) \times 100}{(\text{UI control})}$$

Where, UI stands for ulcer index.

Gastric secretion and the pH of the gastric juice was recorded, Then the contents were subjected to analysis for free and total acidity. Stomachs were preserved in 10% formalin for histopathologically examination[10-11].

Swimming Stress Induced Ulcers In Rats:

Stress ulcers were induced by forced swimming in the glass cylinder (height 45cm, diameter 25cm) containing water to the height of 35cm maintained at 25^{o}C for 3hrs. The albino rats of either sex weighing between 180-200 gm were divided into five groups of six animals each and fasted for 24 hrs with water *ad libitum* prior to experiment. The animals of group I were treated with vehicle (1% Sodium CMC aqueous solution) 1.0 ml/kg p.o. and the animals of group II were treated with standard i.e. Omeprazole (20 mg/kg p.o.). Similarly animals of group III, IV and V were treated with hydro-alcoholic extract orally 100mg/kg, 200mg/kg, 300mg/kg respectively. After the drug treatment animals were allowed to swim in water for 3hrs. The stomach of each animal was removed and the extent of gastric damage was assessed and the percentage protection was calculated as mentioned in the above explained models. Gastric secretion and the pH of the gastric juice were recorded, then the contents were subjected to analysis for free and total acidity. Stomachs were preserved in 10% formalin for histopathologically examination[12].

Gastric Secretion Determination:

The gastric juice was collected and centrifuged for 5 minutes at 2000 rpm and the volume of supernatant was noted. The pH of the gastric juice was recorded by the pH meter. Then the contents were subjected to analysis for free and total acidity. The gastric contents were centrifuged at 1000 rpm for 10 min. 1 ml of supernatant was diluted with 9 ml of distilled water and was

titrated with 0.01 N sodium hydroxide run from microburtte using 3-4 drops of Topfer's reagent as indicator until canary yellow color was observed. Volume of NaOH required was noted. This corresponds to free acidity. Further 2-3 drops of phenolphthalein was added and titrated with NaOH until pink color was restored. This gives total acidity. Free acidity and total acidity is expressed in terms of ml of 0.1 N HCl per 100 gm of gastric contents. This is the same as meq/lit/100gm.[13] To obtained this figure multiply the burette reading obtained from titration by 10.

Statistical Analysis:

The results were expressed as mean ± S.E.M, and statistical significance between treated and control groups was analyzed using of One way analysis of variance (ANOVA), followed by Dunnett's t-test where $P<0.05$ was considered statistically significant.

Histopathological Evaluation:

The gastric tissue samples were fixed in neutral buffered formalin for 24 h. Sections of tissue from stomachs were examined histopathologically to study the ulcerogenic and/ or anti-ulcerogenic activity of *Ficus bengalensis* Linn bark. The tissues were fixed in 10% buffered formalin and were processed using a tissue processor. The processed tissues were embedded in paraffin blocks and about 5-μm thick sections were cut using a rotary microtome. These sections were stained with hematoxylin and eosin using routine procedures. The slides were examined microscopically for Pathomorphological changes such as congestion, haemorrhage, edema, Leucocytic infiltration and Necrosis using an arbitrary scale for the assessment of severity of these changes[14-15].

RESULTS AND DISCUSSION

Phytochemical Screening:

Phytochemical analyses of the Hydro-alcoholic extract of *Ficus bengalensis* bark revealed the presence of flavonoids, tannins, carbohydrates, saponin and steroids.

Acute Toxicity Study:

Single dose (250, 500, 2000 and 5000 mg kg^{-1}) of *F. bengalensis* bark hydro-alcoholic extract administered to albino mice showed no death up to 14 days study period. Even at the highest dose (5000 mg kg-1), there were no physical sings of toxicity as evidenced by normal breathing and the absence of tremors, convulsions, diarrheas, salivation and paralysis in the treated animals. But CNS depression, skin irritation, sedation were noticed up to 3 h after administration of 5000 mg kg^{-1} extract. These observations reveal that the oral LD_{50} of hydro alcoholic bark extract of *F. bengalensis* is greater than 5000 mg kg^{-1} in mice. Observation of animals over the next 14 days showed no adverse effect of treatment.

Indomethacin Induced Ulcers In Rats:

As seen in the Table 1, pretreatment of rats with *Ficus bengalensis* bark hydro-alcoholic extract rendered a dose-dependent protection from indomethacin-induced ulceration, when compared to the indomethacin-induced ulcerated control group, the ulcer index was 23.25 ± 0.9979 and the maximum numbers of ulcers were of the ulcer score 2 and 2.5. Test 1 was found to produce a decrease in ulcer index in the 100 mg/kg dose; the percentage reduction was 36.21**%**. Whereas Test 2 and Test 3 produced more significant ($p<0.01$) decrease in the ulcer index; the percentage reduction being 63.44**%** and 74.19**%**, in the dose of 200mg/kg and 300mg/kg respectively.

The volume of gastric juice in the Test 1, 2 and 3 were less indicating the antisecretory mechanism of the hydro-alcoholic extract of *Ficus bengalensis* Linn. bark. Test 1, 2 and 3 groups showed a significant ($p<0.01$) increase in the pH compared to ulcerated control group. The free acidity and total acidity was also significantly ($p<0.01$) reduced at the dose of Test 1, 2, and 3 as compared to indomethacin--induced ulcerated control group. Omeprazole (20 mg/kg, p.o.) was found to produce significant ($p<0.01$) reduction in ulcer index, the percentage reduction being 68.81**%.** It also reduces the volume of gastric juice, pH, free acidity and total acidity.

Table 1: Anti-ulcer activity of *F. bengalensis* Linn. bark extract in Indomethacin--induced model.

Gr.	Treatment	Ulcer index	pH of gastric juice	Gastric juice (ml)	Free acidity meq/ltr	Total acidity meq/ltr	Protection (%)
I	Vehicle (1%) CMC	23.25 ± 0.9979	2.598 ± 0.1062	2.350 ± 0.1384	26.67±0.9545	49.83 ± 1.493	-
II	Omeprazole (20 mg/kg)	7.250±0.4787**	4.368 ± 0.1295**	1.30 ± 0.07303**	16.00±0.6325**	26.67±1.229**	68.81%
III	HAEFB (100mg/kg)	14.83 ± 0.8724*	3.468 ± 0.1017*	1.567 ± 0.1256*	19.83 ± 0.9458*	33.00 ± 1.673*	36.21%
IV	HAEFB (200mg/kg)	8.50 ± 0.2887**	3.827±0.07936**	1.350 ±0.08466**	15.33 ± 1.054**	27.83±1.621**	63.44%
V	HAEFB (300mg/kg)	6.000±0.4082**	3.998±0.2578**	1.015±.0.06021**	13.83±0.6009**	25.00±1.183**	74.19%

Values are represented as mean ± SEM. Statistical analysis was done by one-way ANOVA followed by Dunnett's t-test. *P < 0.05, **P < 0.01, ***P < 0.001 as compared to ulcerated control. Where, HAEFB means Hydro-alcoholic Extract of *Ficus bengalensis* Linn.

Swimming Stress Induced Ulcers In Rats:

It was observed that in the Swimming stress induced ulcerated control group, the ulcer index was 19.92 ± 1.307 and the maximum numbers of ulcers were of the ulcer score 2 and 2.5. Test 1 was found to produce a decrease in ulcer index in the 100 mg/kg dose; the percentage reduction was 19.67**%.** Whereas Test 2 and Test 3 produced more significant ($p<0.01$) decrease in the ulcer index; the percentage reduction being 34.73**%** and 59.00**%,** in the dose of 200mg/kg and 300mg/kg respectively.

The volume of gastric juice in the Test 1, 2 and 3 were less indicating the antisecretory mechanism of the hydro-alcoholic extract of *ficus bengalensis* Linn. bark. Test 1, 2 and 3 groups showed a significant ($p<0.01$) increase in the pH compared to ulcerated control group. The free acidity and total acidity was also significantly ($p<0.01$) reduced at the dose of Test 1, 2, and 3 as compared to Swimming stress induced ulcerated control group. Omeprazole (20 mg/kg, p.o.) was found to produce significant ($p<0.01$) reduction in ulcer index, the percentage reduction being 55.65**%.** It also reduces the volume of gastric juice, pH, free acidity and total acidity (Table 2).

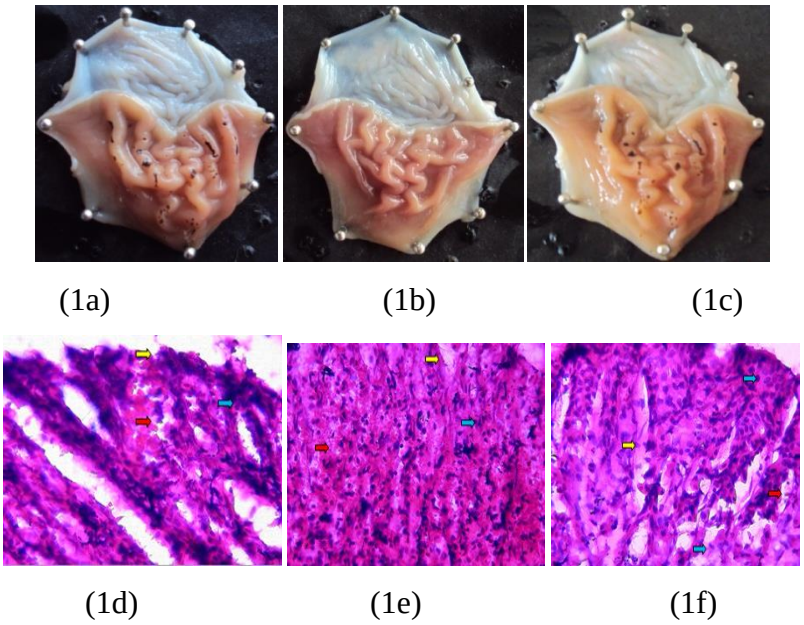
Table 2: Anti-ulcer activity of *F. bengalensis* Linn. bark extract in Swimming stress induced ulcers in rats:

Gr.	Treatment	Ulcer index	pH of gastricjuice	Gastric juice (ml)	Free acidity meq/ltr	Total acidity meq/ltr	Protection (%)
I	Vehicle (1%) CMC	19.92 ± 1.307	2.115±0.01478	1.780±0.08083	27.33±1.430	54.17 ± 2.301	-
II	Omeprazole (20 mg/kg)	8.833±0.6540**	4.202±0.09891**	0.900±0.05164**	17.33±0.5578**	29.33±1.078**	55.65**%**
III	HAEFB (100mg/kg)	16.00 ± 0.3651*	2.827 ± 0.1140*	1.217 ± 0.1014**	21.83 ± 1.046**	38.83 ± 1.537*	19.67**%**
IV	HAEFB (200mg/kg)	13.00±0.6583**	3.435 ± 0.1699**	1.100±0.09309**	20.83±0.9804**	36.00±1.390**	34.73**%**
V	HAEFB (300mg/kg)	8.167±0.7149**	4.083 ± 0.1180**	0.6833±0.07923**	17.17±0.4773**	31.17±0.8333**	59.00**%**

Values are represented as mean ± SEM. Statistical analysis was done by one-way ANOVA followed by Dunnett's t-test. *P < 0.05, **P < 0.01, ***P < 0.001 as compared to ulcerated control. Where, HAEFB means Hydro-alcoholic Extract of *Ficus bengalensis* Linn.

Macroscopical and Histopathological Evaluation:

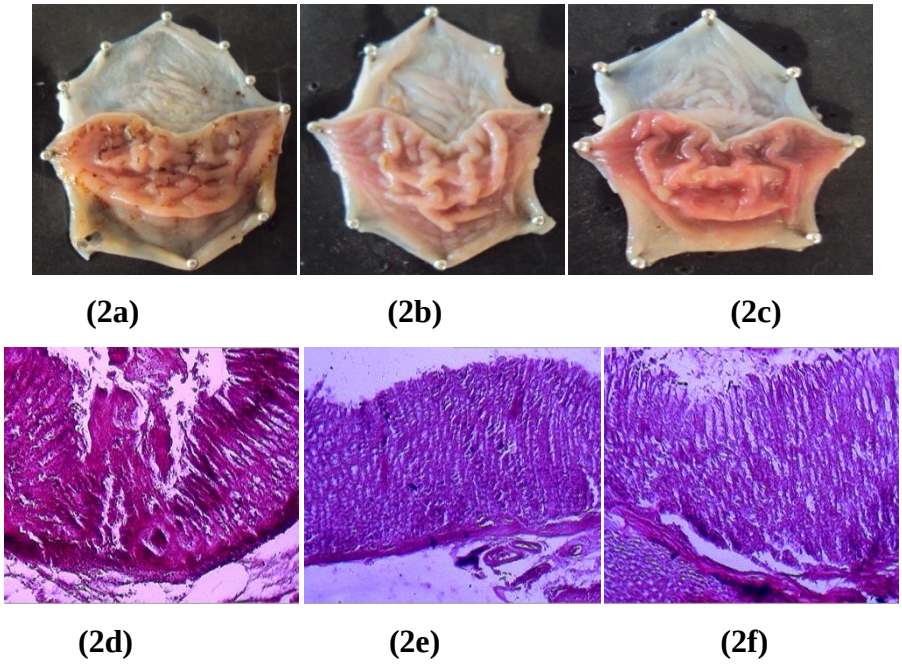
Macroscopical change of indomethacin induced model was shown in figure 1 (1a, 1b, 1c) and in Swimming stress induced model it was shown in figure 2 (2a, 2b, 2c) for ulcerated control, standard Omeprazole 20 mg/kg and HAEFB 300mg/kg respectively. Histopathological changes on ulcerated control group showed the hemorrhages, edema, necrosis leucocytic infiltration, degeneration where as HAEFB (300 mg/kg) and Omeprazole (20 mg/kg) treated groups showed regeneration and prevents the formation of hemorrhage and edema in both indomethacin-induced and swimming stress-induced model, it were shown in figure 1 (1d, 1e, 1f) and figure 2 (2d, 2e, 2f) respectively.



(1a) (1b) (1c)

(1d) (1e) (1f)

Figure 1: Anti-ulcer activity of hydro alcoholic extract obtained from *F. bengalensis* bark in indomethacin-induced model. (1a): Stomach of an ulcer control rat; (1b): Stomach of a rat treated with 20 mg kg^{-1} omeprazole; (1c): Stomach of a rat treated with 300 mg kg^{-1} *F. bengalensis* bark hydro alcoholic extract. Respective histopathological sections are shown down; (1d): Stomach of the ulcer control animal showing mucosa with hemorrhagic erosion, discontinuity in the lining of epithelium cells and significant damage to sub-mucosa; (1e): Stomach of omeprazole 20 mg kg^{-1} treated animals showing normal mucosa with small strophic gland mild hyperplasia and no edema; (1f): Stomach of animals administered with 300 mg kg^{-1} *F. bengalensis* bark hydro alcoholic extract showing normal mucosa with mild hyperplasia and mild edematous sub-mucosa.



(2a) **(2b)** **(2c)**

(2d) **(2e)** **(2f)**

Figure 2: Anti-ulcer activity of hydro alcoholic extract obtained from *F. bengalensis* bark in swimming stress model. (1a): Stomach of an ulcer control rat; (1b): Stomach of a rat treated with 20 mg kg^{-1} omeprazole; (1c): Stomach of a rat treated with 300 mg kg^{-1} *F. bengalensis* bark hydro alcoholic extract. Respective histopathological sections are shown down; (1d): Stomach of the ulcer control animal showing mucosa with hemorrhagic erosion, discontinuity in the lining of epithelium cells and significant damage to sub-mucosa; (1e): Stomach of omeprazole 20 mg kg^{-1} treated animals showing normal mucosa with small strophic gland mild hyperplasia and no edema; (1f): Stomach of animals administered with 300 mg kg^{-1} *F. bengalensis* bark hydro alcoholic extract showing normal mucosa with mild hyperplasia and mild edematous sub-mucosa.

Ulcer has long been recognized as one of the most important gastrointestinal problem. With the ever growing interest in natural medicine, many plants have been screened and reported to be useful in treating and managing ulcer. *F.bengalensis* Linn. have several pharmacological properties including anti-inflammatory and anti-diarrhoeal. In spite of its uses in the traditional medicine against various ailments, bark of this plant has so far not been screened for anti ulcer activity using its hydroalcoholic extracts in indomethacin induced model and swimming stress induced model. We report on the anti-ulcer activity of *F. bengalensis* bark hydro-alcoholic extract for the first time here, as evidenced by its significant inhibition in the formation of ulcers induced by indomethacin (Table 1) and swimming stress (Table 2).

Gastric mucosal damage caused by the indomethacin and related non-steroidal anti-inflammatory drugs result from the inhibition of prostaglandin synthesis via the arachidonic pathway.

Prostaglandin serves protective functions in the stomach by maintaining gastric microcirculation and causing gastric secretion of bicarbonate and mucus, thus, the effect of the herbal drugs against indomethacin–induced gastric ulcer due to it may possess cytoprotective action probably by inhancing prostaglandin synthesis[16].

Swimming stress is one of the best models of stress in rats to induced ulcer. The model provides both emotional stress as well as physical stress to the animals. In this model, ulcers are caused by factors such as increase in gastric motility and gastric acid secretion, decrease in pH, vagal overactivity, mast cell degranulation, decrease in mucosal blood flow, gastric mucus and prostaglandin synthesis as well as generation of the free radicals such as superoxide anion, H_2O_2 and hydroxyl radicals, these induced cell degranulation by increasing peroxidation of cell membrane lipid, causing loss of structural and functional integrity of cell membranes. Accumulation of the H_2O_2 occurs in the mitochondria and cytosol, leads to increase in generation of hydroxyl radicals, thus lead to increase lipid peroxidation level. Ulcer preventive activity of herbal drugs due to it may decrease the lipid peroxidation level[17].

The protective effect was confirmed by histological examination showing prevention of mucosal lesions and sub-mucosal edema. Sub-acute toxicological studies have revealed that the hydro-alcoholic extract of *F. bengalensis* show slight CNS depression for a few hours after treatment at the dose of 5000 mg kg^{-1}. However, there was no sign of toxicity or mortality up to 14 days indicates that the extract is relatively safe.

CONCLUSION

From this study, it is clear that *F. bengalensis* Linn. bark hydro-alcoholic extract have significant anti-ulcer activity in animal models. It has muco-protective activity and gastric antisecretary when compared with that of reference drug Omeprazole. The extract is non-toxic even at relatively high concentrations. The anti-Ulcer activity is probably due to the presence of flavanoids and saponins. Further studies are being carried out to characterize and explore the biological activity of the compounds present in the extract.

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