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Pharmacological evaluation of ethanol extract of *Ficus benghalensis* seeds for antiulcer and antimicrobial efficacy

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The alcoholic extract of the seeds of the plant *Ficus benghalensis* L. has been screened for acute toxicity, gastroprotective effect, antimicrobial efficacy, antioxidant potential and HPTLC analysis.Toxicity study was performed according to the OECD test guidelines and the antiulcer assay was performed using ethanol-induced gastric ulcer model in albino rats. The antimicrobial activity and HPTLC analysis were also performed. The seed extract did not show any sign of toxicity upto dose of 2000 mg/kg body weight. Reductions in the ulcer index and gastric acid volume with increase in the pH of the gastric fluid in extract treated rats proved antiulcer activity. Increased levels of endogenous antioxidant enzymes superoxide dismutase and catalase with a decrease in lipid peroxidation in the extract treated animals demonstrated its antioxidant effect. The zone of inhibition was significant in all the tested microorganisms. HPTLC chromatogram showed a total of 9 peaks at different R_f values and peak area at 366 nm whereas seven peaks were observed at 254 nm. The number of peaks indicates the presence of constituents in the extract. Pre-treatment with *F. benghalensis* seed extract showed appreciable antiulcer activity that might be attributed to its antioxidant potential. The extract also showed approciable activity against the bacteria and fungi tested. The extract containing phytoconstituents must have contributed to this property.

Keywords: Acute oral toxicity, Antiulcer activity, Antioxidant activity, Antimicrobial activity, *Ficus benghalensis* seed extract.
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Introduction

A peptic ulcer is an important cause of morbidity and mortality throughout the world affecting the lives of millions of people in their everyday life. In the United States, approximately 4 million people have peptic ulcers and 3, 50,000 new cases are diagnosed each year. Around 1, 80,000 patients are hospitalised yearly, and about 5000 people die each year as a result of peptic ulcer disease[1]. The incidence of peptic ulcers has been estimated at around 1.5 % to 3 %[2]. The lifetime likelihood of developing peptic ulcer is about 10 % for males and 4 % for females[1]. Peptic ulcer deaths in India reached 1.2 % of total deaths. The age-adjusted death rate is 12.37/1, 00,000 of the population, ranks India at 5 in the world[3]. Gram-negative bacteria *H. pylori*, non-steroidal anti-inflammatory drugs, emotional stress, alcohol abuse and smoking are the principal etiological factors associated with peptic ulcer. Though most of the ulcers are treated by synthetic drugs such as antacids, cimetidine, ranitidine, omeprazole, etc. they result in some side effects[4]. Hence an attempt has been made to find out a suitable remedy for this malady from natural sources. In the traditional *Ayurvedic* medical practices, the seed of *Ficus benghalensis* is used to treat ulcers[5]. As no studies have been carried out so far on the seeds of this plant, they were considered for screening in experimental rats. This study also focused on the evaluation of *F. benghalensis* seeds against few pathogenic microbes.

F. benghalensis L. (syn. *F. indica*) belongs to the family Moraceae and is commonly known as banyan tree. This tree is a native of Indian subcontinent spreading from Burma to Malaysia. Various parts of *F. benghalensis* are used in *Ayurveda* and *Siddha* systems of medicine to treat a wide variety of diseases that include diabetes, diarrhoea and ulcer[6].

Materials and Methods

Chemicals

All the chemicals used were of analytical grade and obtained from E. Merck limited, India and Hi-Media laboratories, Mumbai, India.

Plant material

F. benghalensis seeds were collected from Thennampattu village of Thiruvannamalai district,

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Tamil Nadu, India and authenticated by Dr P. Jayaraman, Director, Plant Anatomy Research Center (PARC), Chennai and a voucher specimen was deposited at the herbarium of PARC for future reference [PARC/2014/2276]. About 500 g of shade-dried seeds were coarsely powdered and extracted with 95 % ethanol in a Soxhlet apparatus. After 24 h, the solvent was distilled off over a boiling water bath, and the final traces of ethanol were removed on rotary evaporator. The final brown coloured crude extract obtained has been designated as FBE (17 g) and was stored in an airtight container for further studies.

Experimental animals

Thirty male and female Wistar Albino rats (150-200 g) were used for the study. The animals were housed in polypropylene cages in a well-ventilated room (air cycles: 15/min; recycle ratio: 70:30) under an ambient temperature of 22±3 °C and 40–65 % relative humidity, with a 12 h light/dark cycle. They were provided with feed (M/s. Provimi Animal Nutrition Pvt. Ltd, India) as well as purified water ad libitum. All experiments were performed as per CPCSEA guidelines with the approval of Institutional Animal Ethics Committee (IAEC), Saveetha University, Chennai (Approval no: SU/BRULAC/RD/022/2014) where the study has been conducted.

Acute toxicity study

Acute oral toxicity study was performed according to the OECD test guideline 423– Acute toxic class method[7]. Six rats were divided into two groups of 3 animals each. A single dose of 2000 mg/kg body weight of FBE was administered orally to the animals and observed for lethality and any abnormal clinical signs for 24 h and the following 13 days. Body weight was recorded before dosing and after that once a week till completion of the experiment. Gross pathological changes were also observed at the end of the experiment.

Antiulcer study

Twenty four animals were selected for the study and fasted for 18 hrs before the experiment. They were divided into four groups each containing six rats. The groups were pretreated for 10 days as follows: Group I- Ulcer control (Vehicle; 5 mL/kg, p.o.), Group II- Standard drug (Ranitidine; 100 mg/kg, p. o.), Group III- FBE (100 mg/kg, p. o.), and Group IV- FBE (200 mg/kg, p.o.). FBE was suspended in 0.5 % carboxyl methyl cellulose and administered to the animals. On the 10th day, one hour after a final dose of treatment, the gastric ulcer has been induced in each rat in all groups by administering 95 % ethanol (5 mL/kg)[8], and after one h, all the animals were sacrificed. Abdomens of the animals were opened, and oesophagal end of the stomachs was tied and taken out. The gastric content was collected and centrifuged. The gastric acid volume and pH of gastric fluid were determined[9]. The stomach was then incised along the greater curvature and observed for ulcers. Ulcers were scored, and the ulcer index was determined[9]. Then, the stomach tissue were processed for antioxidant parameters such as lipid peroxidation, superoxide dismutase and catalase[10].

Histopathological studies

Tissues of the stomach were collected, blotted to free from blood, fixed in 10 % neutral buffered formalin for 48 h, trimmed and processed for paraffin embedment and 5 µm thicknesses of sections were stained with haematoxylin and eosin for histopathological examination[11] and interpreted accordingly.

Determination of antimicrobial activity

Antimicrobial activity of FBE was tested using disc diffusion assay[12]. Two bacterial cultures (*Vibrio cholerae* and *Klebsiella pneumonia*) and one fungal culture (*Candida albicans*) were used in this study. All the cultures were obtained from Royal Bioresearch Centre, Velachery, Chennai. The cultures were stored on nutrient agar slants at 4 °C and were subcultured on a nutrient agar medium before antimicrobial testing. Average of triplet readings for each microorganism was recorded.

HPTLC analysis

CAMAG-HPTLC system of Switzerland with a Linomat 5 sample applicator was used to obtain HPTLC fingerprinting. HPTLC fingerprint profile of FBE was developed to confirm the occurrence of different phytochemicals by using the method of Aftab Ahmad *et al*.[13]

Statistical analysis

Data obtained were expressed as Mean±SEM of six replicates and subjected to One way ANOVA followed by Dunnett's multiple comparison tests using Graph Pad Prism 5.03. Values were considered statistically significant at $p < 0.05$.

Results and Discussion

The medicinal values of plants lie in their phytochemicals. Phytochemicals have antioxidant or

hormone-like effects which help in fighting against many diseases including cancer, diabetes, ulcer and arthritis[14]. In a previous study by the author, on the phytochemical screening of FBE extract revealed the presence of various components such as carbohydrates, phenols, tannins, flavones, saponins, steroids, quinones, terpenoids, coumarins, cardiac glycosides and alkaloids among which phenols, tannins and flavones were the most prominent ones[15]. A study by Bors and Michel[16] demonstrated that certain terpenoids, steroids and phenolic compounds (tannins, coumarins and flavonoids) have protective effects due to their antioxidant properties.

HPTLC fingerprinting of FBE extract was carried out to confirm the presence of various phytoconstituents in the extract. The chromatogram (Fig. 1) showed a total of nine peaks at different R_f values at 366 nm whereas seven peaks were observed at 254 nm. The number of peaks in the FBE extract and their R_f is summarised in Table 1. A number of peaks indicate the presence of constituents in the extract. Presence of major phytoconstituents in the FBE extract makes it a potential candidate for further investigation.

In the present study, an investigation was made to check whether the plant has acute toxicity. After administration of FBE extract, the animals were observed individually for four hour and following 14 days to check mortality and their behavioural pattern. Results showed no deaths or abnormal clinical signs or remarkable body weight changes were observed in the experimental animals. This shows the non-toxic nature of seed extract. No gross pathological observation was recorded. This further confirmed the extract to be safe and the test drug was found to be safe up to a dose of 2000 mg/kg b.w.

The anti-ulcer effect of FBE extract was evaluated using ethanol-induced gastric ulcer model. Ethanol is considered one of the agents that induce gastric ulcers. The effect of ethanol on the gastric mucosa may occur as a result of stasis in gastric blood flow, which contributes to the development of the hemorrhagic and necrotic aspects of tissue injury. The occurrence of these ulcers, which is predominant in the glandular part of the stomach, was reported to stimulate the formation of reactive oxygen species (ROS), resulting in damage to rat gastric mucosa[17]. The results revealed that the oral administration of absolute ethanol produced severe ulceration in the stomach of the control rats. However, treatment with ranitidine at the dose of 100 mg/kg and FBE extract at the doses of 100 and 200 mg/kg prior to ethanol administration exhibited significant (p <0.001) inhibition with 90.9 %, 81.8 % and 86.3 % respectively (Table 2 and Fig. 2). Among the tested doses, better result was obtained with FBE extract at 200 mg/kg as compared to the standard drug, ranitidine. In *in-vivo* antioxidant studies, FBE extract at the dose of 200 mg/kg showed a significant reduction in lipid peroxidase and increase in superoxide dismutase, catalase and glutathione levels as compared to the standard drug, ranitidine (Fig. 3) suggesting the ability of seed extract in the protection of gastric mucosa against free radical-mediated tissue

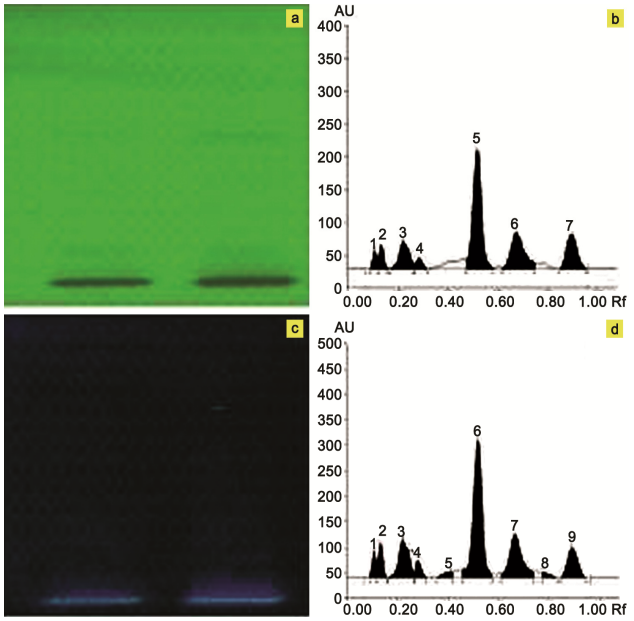

Fig.1 — HPTLC photograph and Chromatogram of FBE extract. a) HPTLC photograph at 254 nm, b) Chromatogram at 254 nm, c) HPTLC photograph at 366 nm, d) Chromatogram at 366 nm, Track 1: 2 µLl of FBE extract, Track 2: 4 µL of FBE extract.

Table 1 — HPTLC fingerprinting profile of FBE extract

Wavelength	Solvent systems	No. of peaks	R_f values	Percentage peak area
254 nm	Toluene: ethyl acetate (9.3:0.7)	7	0.12, 0.16, 0.26, 0.32, 0.58, 0.75, 0.95	2.97, 4.66, 11.35, 2.93, 42.75, 20.16, 15.18
366 nm	Toluene: ethyl acetate (9.3:0.7)	9	0.12, 0.16, 0.27, 0.32, 0.42, 0.58, 0.74, 0.83, 0.94	3.41, 5.58, 13.1, 3.36, 1.94, 42.16, 18.6, 1.38, 10.48

All values are average of triplet readings

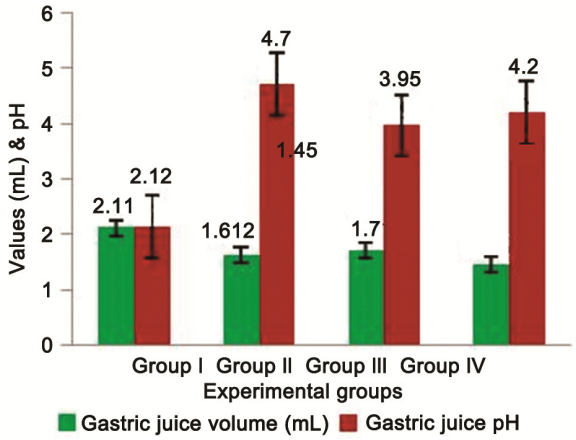

Fig.2 — Effect of FBE extract on volume and pH of gastric content in ethanol-induced gastric ulcer in rats. All values are Mean ± SEM for six rats. * indicates significant at p <0.05 compared to group I. Group I: Ulcer control, Group II: Ranitidine treated (100 mg/kg), Group III: FBE treated (100 mg/kg), Group IV: FBE treated (200 mg/kg).

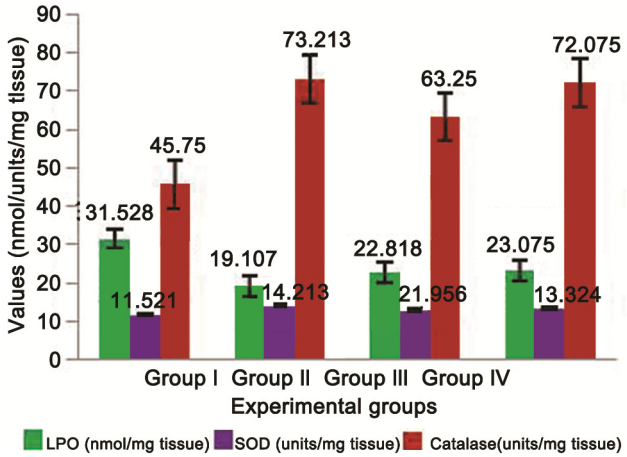

Fig.3 — Effect of FBE extract on antioxidant enzymes in the stomach of ethanol-induced gastric ulcer in rats. All values are Mean±SEM for six rats. * indicates significant at p <0.05 compared to group I. Group I: Ulcer control, Group II: Ranitidine treated (100 mg/kg), Group III: FBE treated (100 mg/kg), Group IV: FBE treated (200 mg/kg).

Table 2 — Effect of FBE extract on ulcer index and % gastroprotection in ethanol-induced gastric ulcer in rats

Groups	Treatment (mg/kg)	Ulcer index	% Gastro protection
I	Ulcer control (5 mL/kg)	2.75±0.316	-
II	Ranitidine (100 mg/kg)	0.25±0.025*	90.9±0.614*
III	FBE (100 mg/kg)	0.50±0.050*	81.8±0.312*
IV	FBE (200 mg/kg)	0.375±0.032*	86.3±0.229*

All values are Mean±SEM for six rats. * indicates significant at p <0.05 compared to group I

injury. A study by Patil and Rita Saini[18] demonstrated that stem bark extract of *F. benghalensis* could protect the gastric mucosa against ethanol challenge by the prostaglandin-like mechanism. Similar findings exist in the literature, where plant extracts have been shown to prevent gastric mucosal ulceration in rats using ethanol model[19]. The study by Rajasekaran[20] demonstrated that *Kigelia pinnata,* a species in the family Bignoniaceae significantly reduced the ulcer lesion index produced by ethanol in a dose-dependent manner. Edema, cellular debris and damaged mucosal epithelium were found in ulcerated stomach membranes (Plate 1). Protection against these histopathological changes by FBE in pre-treated rats was observed, similar to the result of ranitidine (Plate 2).

Additionally, the extract inhibited the growth of few harmful microorganisms. Almost all the microorganisms were susceptible to the extract though in different concentrations. The extract possessed good inhibitory activity against *V. cholerae*

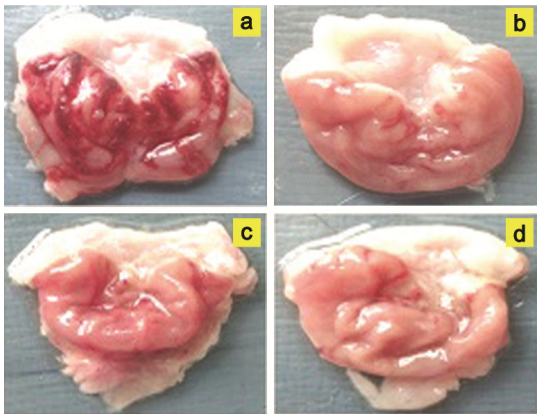

Plate 1 — Gross anatomy of ethanol-induced gastric mucosa ulcer in rats. All values are mean for six rats**.** a) Ulcer control: marked ulcers along with hemorrhagic streaks and mucosal damage were observed, b) 100 mg/kg ranitidine: mild injuries were observed in the gastric mucosa as compared to the ulcer control group, c) 100 mg/kg FBE extract**:** moderately reduced gastric mucosal damage and ulcers were observed, d) 200 mg/kg FBE extract: significantly reduced gastric mucosal damage and ulcers were observed as compared to the ulcer control.

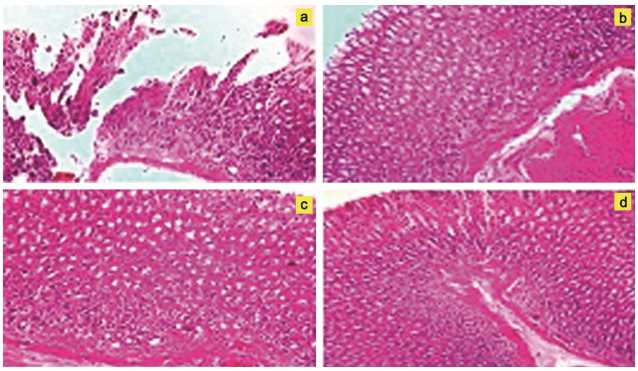

Plate 2 — Histopathology of rat stomach tissue samples in ethanol-induced ulcer model. All values are mean for six rats. a) Ulcer control: demonstrated mucosal degeneration, ulceration and migration of numerous inflammatory cells throughout the section, b) 100 mg/kg ranitidine: no significant change in histopathology and in turn revealed regeneration of structure and prevention of hemorrhage and edema, c) 100 mg/kg FBE extract**:** exhibited moderate regeneration, d) 200 mg/kg FBE extract: displayed significant regeneration of mucosal layer and expressively prevented the development of hemorrhage and edema.

(18 mm) at 1000 µg/mL (Table 3 and Plate 3). This evidence opens up possibilities to the fact that the extract contains compounds that may act by synergism or additive effect which in turn is responsible for its pharmacological activity[21]. The results showed a good correlation between the reported uses of *F. benghalensis* in traditional medicine against infectious diseases. A study by Agarwal *et al.*[22] demonstrated that the flavonoid

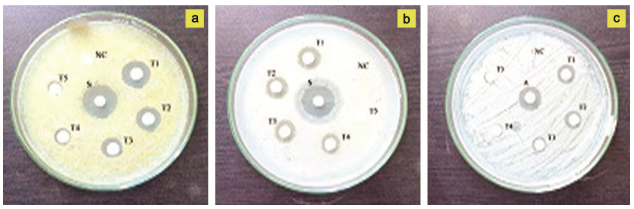

Plate 3 — Antimicrobial activity of FBE extract using disc diffusion assay. a) *V. cholerae*, b) *K. pneumonia*, c) *C. albicans*. T1: 1000 µg/mL, T2: 500 µg/mL, T3: 250 µg/mL, T4: 125 µg/mL, T5: 62.5 µg/mL, NC: Negative control 1 % DMSO, S: Streptomycin, A: Amphotericin B, *V. cholera: Vibrio cholerae, K. pneumonia: Klebsiella pneumonia, C. albicans: Candida albicans.*

Table 3 — Antimicrobial activity of FBE extract in different concentrations

Microorganisms	Zone of inhibition (mm)							
	1000 µg/mL	500 µg/mL	250 µg/mL	125 µg/mL	62.5 µg/mL	DMSO 1%v/v	Amphotericin B 10 µg/mL	Streptomycin 10 µg/mL
V. cholerae	18	14	10	7	3	-	-	20
K. pneumoniae	17	15	14	10	-	-	-	23
C. albicans	12	10	7	3	-	-	15	-

All values are average of triplet readings

component of *Glycyrrhiza glabra* root possesses antimicrobial activity, beneficial in the treatment of peptic ulcer and also effective against gastric and duodenal ulcers.

Conclusion

In this study, the extract exhibited strong protection against characteristic lesions produced by ethanol administration. This antiulcer effect of FBE extract may be due to its antioxidant and gastroprotective effect of the ingredients present in it and thereby confirming the traditional medical claim in the treatment of gastric ulcer. The demonstration of antimicrobial activity is an indication that *F. benghalensis* seeds can be a source of bioactive substances. The extract containing phytoconstituents must have contributed to its antimicrobial activity. Therefore, further experiments should be undertaken to identify which of the phytoconstituents and mechanisms are involved in the actions illustrated by the results.

Conflict of interests

The authors declare that they have no conflict of interest.

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