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Anti-ulcer activity of *Ficus religiosa* leaf ethanolic extractMarslin Gregory<sup>1,2</sup>, B. Divya<sup>3</sup>, Revina Ann Mary<sup>4</sup>, M. M. Hipolith Viji<sup>5</sup>, V. K. Kalaichelvan<sup>1</sup>, V. Palanivel<sup>1,3\*</sup><sup>1</sup>Department of pharmacology, Annamalai University, Chidambaram, India<sup>2</sup>Centre for the Research and Technology of Agro-Environment and Biological Sciences (CITAB), Department of Biology, University of Minho, Portugal<sup>3</sup>Padmavathi College of Pharmacy and Research Institute, Periyannahalli, Dharmapuri, Tamilnadu, India<sup>4</sup>Department of Nursing Science, A.J College of Nursing, Chennai, Tamilnadu, India<sup>5</sup>Department of Phytochemistry, Natural Remedies Research lab, Bangalore, Karnataka, India

## ARTICLE INFO

## Peer reviewer

Dr. Vinoth KM Khandelwal, Department of Translational Pharmacology, Postdoctoral researcher, Consorzio Mario Negri Sud, Santa Maria Imbaro, Italy.  
E-mail: Vinoth@negrisud.it

## Comments

The study has been well planned and executed. The study provides evidence for the anti-ulcer activity of the ethanolic extract of *F. religiosa* leaf.

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## ABSTRACT

**Objective:** To evaluate the anti-ulcer activity and acute toxicity of *Ficus religiosa* (*F. religiosa*) leaf ethanolic extract in animal models. **Methods:** Anti-ulcer activity of *F. religiosa* ethanolic extract (250 and 500 mg/kg body weight) was studied on stress induced ulcer animal models. Ranitidine was used as standard. The anti-ulcer activity of *F. religiosa* was evaluated with the help of ulcer area and histopathological examination. Preliminary phyto-chemical screening and acute toxicity studies of *F. religiosa* also carried out. **Results:** Results showed that the extract treatments prevented ulcer area and gastric secretion in a dose-dependent manner. Administration of 2000 mg/kg extract did not show any acute toxicity in albino mice. Preliminary phytochemical analysis identified the presence of flavonoids in the ethanolic extract of *F. religiosa*. **Conclusions:** The extract is non-toxic even at relatively high concentrations. The anti-ulcer activity is probably due to the presence of flavonoids.

## KEYWORDS

*Ficus religiosa*, Flavonoids, Ethanolic extract, Ulcer area, Ranitidine

## 1. Introduction

Ulcer has long been recognised as one of the most important problem in developing countries. About 70% population in developing countries relies on traditional medicine for their primary health care needs[1]. With the ever growing interest in natural medicine, many plants have been screened and reported to be useful in treating and managing ulcer.

*Ficus religiosa* (*F. religiosa*) Miq. (Moraceae) is an important traditional medicinal plant distributed throughout India, mostly near to the Indian temple for the spirituality. It has several vernacular names including peepal tree and arasa maram. The bark of the plant contains carbohydrates, flavonoids, aminoacids, steroids, saponins and tannins etc. are present[2]. Bark and leaf extract of this plant is being used in the traditional medicine. Bark of the plant is used as gonorrhoea, diarrhea, dysentery, leucorrhoea, menorrhagia, vaginal and other urogenital disorders haemorrhoids, ulcers and gastrohelcosis. It is also useful in inflammation, burning sensation, anti-bacterial, analgesic, anti-diabetic and anti-oxidant[3]. In the present investigation, we studied the anti-ulcer (ulcer-preventive) and acute toxicological effects of *F. religiosa* ethanolic leaf extract.

## 2. Materials and methods

## 2.1. Plant material

Fresh leaves were collected from a *F. religiosa* plant growing at the Padmavathi College botanical garden. The plant was identified taxonomically and authenticated by Dr. Jayaraman (National Institute of Herbal Science, Tamilnadu, India). Leaves were dried under shade and powdered and stored in an airtight container. For extraction, 250 g of dried leaf powder was loosely packed in the thimble of soxhlet apparatus and extracted with ethanol for 18 h at 55 °C. The extract was air dried at 25–30 °C and weighed. For oral administration, extract was dissolved with the help in 10 mL phosphate buffer saline (PBS) at different concentrations.

## 2.2. Phytochemical screening

The ethanol extract of the *F. religiosa* was evaluated for the presence of flavonoids, tannins, alkaloids, saponins, glycosides and sterols/triterpenes using methods of Brain *et al*[4].

## 2.3. Animals

Wistar rats (180–200 g) and male albino mice (20–25 g)

\*Corresponding author: Palanivel V, M.Pharm (PhD), Assistant Professor, Department of Pharmacology, Padmavathi college of Pharmacy and Research Institute, Peryannahalli, Dharmapuri Dist, Pin:No: 635205, Tamilnadu, India.  
Tel: +91-9894269874  
E-mail: pharma.palani@gmail.com

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were obtained from the animal house, Padmavathi College of Pharmacy and kept in standard environmental conditions. They were fed with rodent diet and water *ad libitum*. Experiments were carried out in accordance with CPCSEA guidelines and the study was approved by Institutional animal ethical committee.

### 2.4. Anti-ulcer activity of *F. religiosa* ethanolic extract

#### 2.4.1. Stress induced ulcers (cold water immersion method)

Stress ulcers were induced by forcing the Wistar albino rats of either sex to swim in the glass cylinder containing water to the height of 35 cm maintained at 25 °C for 3 h. Animals were fasted for 24 h prior to the experiment and divided into three groups each group consist of six animals. Group 1 received 1.0 mL/kg *p.o.* as vehicle control; Group 2 received 50 mg/kg, *p.o.* ranitidine as standard control; Groups 3 and 4 were respectively received 250 and 500 mg/kg, *p.o.* of *F. religiosa* ethanolic extract. After the drug treatment animals were allowed to swim in cold water for 4 h. After this the animals were killed with high dose of anaesthetic ether. Each stomach was opened along the greater curvature and examined macroscopically for gastric erosions under a dissecting microscope (10×). Gastric juice collected into centrifuge tubes and centrifuged at 1000 r/min for 10 min and the volume was noted. The pH of the gastric juice was recorded by pH meter and the gastric content is subjected for analysis of free and total acidity. The ulcer area (UA) was calculated. The percentage of protection availed to the animals through various treatments was calculated[5,6].

### 2.5. Histopathology

After collecting the gastric contents and measuring the UA, small pieces of stomachs from each group were fixed in 10% (v/v) formalin and subsequently embedded into paraffin wax. Sections of 5 µm thick were cut in a microtome and mounted on the glass microscope slides using standard techniques. After staining with hematoxylin–eosin, the sections were examined under light microscope and photographed[7].

### 2.6. Acute toxicity study

Four groups (*n*=6) of male albino mice were used to study the acute toxicity of *F. religiosa* extract administration. Animals were fasted overnight and administered with ethanolic extract (50, 500 and 2000 mg/kg). A group of animals which received equal volume of PBS served as control. Changes in the animal behaviour were noted before and after administration for 24 h. Treated animals were further observed for up to 14 d for any signs of toxicity[8].

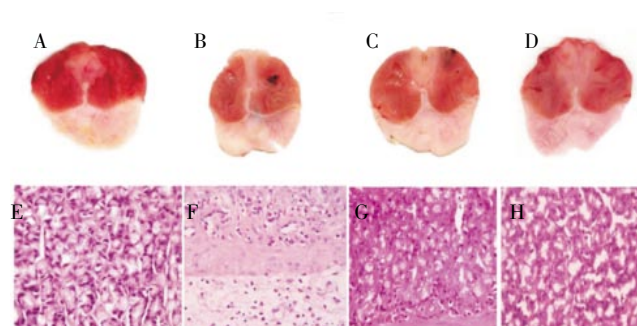
### 2.7. Statistical analysis

The results are expressed as mean±SEM. Statistical difference

between means were determined by one-way ANOVA followed by Dunnett's *post hoc* test (Del/was) to analyze and compared data with *P*>0.05 as the limit of significance.

## 3. Results

Water immersion stress was one of the best models of stress in rats to induce ulcer[9]. The model provided both emotional stress as well as physiological stress to the animal. We have studied the anti-ulcer activity of *F. religiosa* leaf ethanolic extract on cold stress induced animal model. The results of the present study showed that *F. religiosa* leaf ethanolic extract possessed anti-ulcer activity, as evidenced by its significant inhibition in the formation of ulcers induced by stress induced ulcer models (Table 1). The animals treated with *F. religiosa* leaf extract 500 mg/kg did not produce any significant change in the volume of gastric secretion as compared to rats which received standard drug ranitidine. Microscopical examination of the stomachs removed from animals that were not treated with either ranitidine or *F. religiosa* showed complete ulceration (Figure 1). However, a preventive effect against ulceration (in terms of UA) was noticed in animals treated with ranitidine, 250 mg/kg and 500 mg/kg ethanolic extract of *F. religiosa*.



**Figure 1.** Anti-ulcer activity ethanolic extract obtained from *F. religiosa* leaves.

A: Stomach of an ulcer control rat showing larger ulcer area; B: Stomach of a rat treated with ranitidine; C: Stomach of rats treated with extract 250 mg/kg; D: Stomach of rat treated with 500 mg/kg; E: Stomach of the ulcer control; F: Stomach of a rat treated with ranitidine; G: Stomach of 250 mg/kg *F. religiosa* extract treated animals showing normal mucosa with small strophic gland mild hyperplasia and no edema; H: Stomach of animals administered with 500 mg/kg of *F. religiosa* leaf ethanolic extract showing normal mucosa with mild hyperplasia and mild edematous submucosa.

Histopathology of stomach showed that animals that have received 500 mg/kg extract significantly reduced gastric lesion formation and submucosal edema similar to the ranitidine treated animals. Photomicrographs revealed that the mucosa of ulcer control animals have hemorrhagic erosion, discontinuity in the lining of epithelium cells and significant damage in submucosa. Normal mucosa with small strophic gland, mild hyperplasia and no edema were observed for animals treated with ranitidine. Similarly, mucosa of animals treated with 250 mg/kg and 500 mg/kg of *F. religiosa* ethanolic extract was

**Table 1**

Anti-ulcer activity of *F. religiosa* ethanolic extract.

Treatment	Dose (mg/kg)	Gastric volume (mL)	Free acidity (m/eq) 100 g	Total acidity (m/eq) 100 g	Ulcer index (mm <sup>2</sup> /rat)	Protection (%)
Control	1	5.28±0.12	37.33±1.80	74.13±1.70	4.20±0.21	–
Ranitidine	50	3.92±0.71	14.40±0.36	27.46±0.76	1.69±0.11	59.76%
Treatment 1 ( <i>F. religiosa</i> )	250	4.04±0.07*	24.66±0.66	68.06±0.72	2.30±0.16	45.23%
Treatment 2 ( <i>F. religiosa</i> )	500	4.32±0.06*	22.60±0.93*	39.83±1.31*	1.62±0.11*	61.42%

Gastric volume and UA of treated and control animals represented as mean±SEM. Values in the column identified by different letters are significantly different (one-way analysis of variance, *P*<0.001). Values with an asterix (\*) are significantly different from the ulcer control (*P*<0.001). –: not applicable.

normal with mild hyperplasia. However, slightly edematous submucosa was noticed in the latter. Acute toxicological studies have revealed that the ethanolic extract of *F. religiosa* showed slight CNS depression for a few hours after treatment (Table 2). However, even at the dose of 2000 mg/kg, there was no sign of toxicity and mortality up to 14 d.

**Table 2**

Changes in the animal behaviour after administration of *F. religiosa* ethanolic extract.

Symptoms	Time after administration (h)					
	2	3	5	7	12	24
Respiration	-	-	-	-	-	-
Writhing	-	-	-	-	-	-
Tremors	-	-	-	-	-	-
Convulsion	-	-	-	-	-	-
Salivation	-	-	-	-	-	-
Diarrhoea	-	-	-	-	-	-
Mortality	-	-	-	-	-	-
Hind limb Paralysis	-	-	-	-	-	-
Sedation	+	-	-	-	-	-
Skin irritation	+	-	-	-	-	-
Eye irritation	-	-	-	-	-	-
CNS Depression	+	-	-	-	-	-

## 4. Discussion

Although the mechanism of ulcer prevention by this extract is not clear, phytochemicals like flavonoids present in the extract might play an important role. In our earlier study, the similar results of *F. arnottiana* methanolic extract were obtained and we have reported [10]. The phytochemical screening confirms the presence of carbohydrate, proteins, tannins, flavonoids and phenolic compounds. Compounds like flavonoids are of particular interest, as they have been reported for their anti-ulcerogenic activity and gastric protection. From this study, it is clear that *F. religiosa* leaf extract have significant anti-ulcer activity in animal models. It has gastric antisecretory when compared with that of reference drugs ranitidine. The extract is non-toxic even at relatively high concentrations. The anti-ulcer activity is probably due to the presence of flavanoids. Further studies are being carried out to characterise and explore the biological activity of the compounds present in the extract.

## Conflict of interest statement

We declare that we have no conflict of interest.

## Acknowledgements

We would like to thank Animal house and Animal ethical committee, Padmavathi College of Pharmacy for their help during the experiments.

## Comments

### Background

In this study, authors have evaluated the “anti-ulcer activity of *F. religiosa* leaf ethanolic extract”. The plant has been traditionally used for inflammation, analgesic, ulcers, etc.

### Research frontiers

Ulcer is one of the important problems in the developing as well as developed countries. Even though many drugs are available to treat ulcer, many of these do not fulfill the requirements and have side effects. In this work, authors tested a natural medicine for treating ulcer. Authors provided evidence

for reduced toxicity and anti-ulcer activity of *F. religiosa* leaf ethanolic extract. The results were clear and interesting. The results presented in this article would be useful for finding new drug for ulcer.

### Related reports

Water immersion stress induced ulcer in animals is one of the established models to study the anti-ulcer activity. *F. religiosa* ethanolic extract does not show any signs of toxicity even at 2000 mg/kg. In the anti-ulcer study, the animals treated with 500 mg/kg extract did not display any significant changes in the volume of gastric secretions, as compared to the animals treated with standard drug ranitidine. Furthermore, it is confirmed by microscopical and histopathological examinations.

### Innovations and breakthroughs

Authors tested a traditionally used natural source *F. religiosa* for anti-ulcer activity. There is no known study reporting the anti-ulcer activity of *F. religiosa* leaf ethanolic extract. It is shown to be safe even at higher doses, observed from acute toxicity study. In terms of anti-ulcer activity, *F. religiosa* extract showed very good effects on water immersion ulcer induced animal model. The preliminary phytochemical study shows the presence of flavonoids.

### Applications

In the recent years, there has been much interest in natural medicine derived from traditional knowledge of plant pharmacological properties. *F. religiosa* extract has very good anti-ulcer activity. The results presented direct towards the development of a potential new drug for the treatment of ulcer.

### Peer review

The study has been well planned and executed. The study provides evidence for the anti-ulcer activity of the ethanolic extract of *F. religiosa* leaf.

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