Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Study of anti ulcer activity of *Ficus religiosa* L. on experimentally induced gastric ulcers in rats

Sarmistha Saha[1*], Gagan Goswami[2]

[1]Department of Zoology, Gujarat University, India
[2]NIPER Ahmedabad, India

ARTICLE INFO

ABSTRACT

Objective: To investigate the gastroprotective activity of hydroalcoholic extract leaves of *Ficus religiosa* (*F. religiosa*) in different experimental models of gastric ulcer in rats. **Methods:** The hydroalcoholic extract leaves of *F. religiosa* were studied at two dose levels (250 and 500 mg/kg, oral) in rats against absolute ethanol (0.2 mL oral), aspirin (200 mg/kg) and pyloric ligation induced gastric ulcer. Ranitidine (50 mg/kg, oral) was used as a standard drug. Mean ulcer indices and oxidative stress were measured. Phytochemical tests and acute toxicity tests were also carried out. **Results:** Administration of *F. religiosa* to rats significantly decreased the ulcer index value when compared with the control treated group. Ranitidine (50 mg/kg, oral) also produced a significant decrease the ulcer index value when compared with the control treated group. Phytochemical analysis revealed the presence of tannins, sterols, saponins, flavonoids, carbohydrates and proteins. **Conclusions:** The results suggest that the leaves of the *F. religiosa* possess significant anti ulcer activity.

1. Introduction

Gastric ulcer is an imbalance between damaging factors within the lumen and protective mechanisms within the gastro duodenal mucosa. The excess gastric acid formation by prostaglandin includes both increases in mucosal resistance as well as a decrease in aggressive factors, mainly acid and pepsin[1]. In Ayurveda, an ancient system of Indian medicine, gastric disorders are classified as sula, parinamasula and amlapitta which correspond to clinical conditions of peptic ulcer and functional dyspepsia[2,3]. In current medicine, ulcer therapy mainly includes H_2 receptor antagonists, proton pump inhibitors or cytoprotective agents such as sucralfate. Oxygen derived free radicals have been implicated in the pathogenesis of a wide variety of clinical disorders and gastric damage[4].

Ficus religiosa (*F. religiosa*) L. belongs to the family *Moraceae*, has many medicinal properties. *F. religiosa* is grown throughout India and is referred as papal in hindi and asvattha in sanskrit. The leaves are used in traditional medicine for the treatment of ulcer and wounds. The bark is used for the treatment of skin diseases, scabies, ulcers, as astringent and as tonic. The fruits and tender buds are used as laxatives. The root bark is used for stomatitis, ulcers and promotes granulations. Besides being used in traditional medicine, its pharmacological properties have not yet been studied. This study was therefore designed to investigate the efficacy of an extract of *F. religiosa* in gastric ulcer with pylorus ligation, ethanol and aspirin induced ulcers in a rat model.

2. Materials and methods

2.1. Plant material

The leaves of *F. religiosa* were collected from Ahmedabad, Gujarat during September 2008. The leaves were shade dried, powdered and stored in airtight container until further use. The leaves were extracted using 75% methanol (hydroalcoholic) as a solvent in a soxhlet apparatus until complete extraction.

2.2. Experimental animals

Healthy Sprague−Dawley rats of either sex weighing between 250−275 g were used for the study. They were kept in the departmental animal house at (26±2) ℃ and relative humidity 44%−56%, light and dark cycles of 10 and 14 h, respectively, for 1 week and during the experiments.

*Corresponding author: Sarmistha Saha, Department of Zoology, Gujarat University, India.
Tel: 919824835780
E−mail: saha.786@hotmail.com

Animals were provided with standard rodent rat pellet diet and food was withdrawn 18–24 h before the experiment through water was allowed ad libitum.

2.3. Preliminary phytochemical analysis

The extracts were subjected to preliminary phytochemical screening[5].

2.4. Acute toxicity test

The acute toxicity (LD_{50}) of the extract of *F. religiosa* was determined in albino mice by the method of Dede and Dogara[6] using the oral route.

2.5. Ethanol induced gastric lesions

Animals were divided into thirteen groups. Group I served as control and received only olive oil. Animals of group 2 received absolute ethanol (0.2 mL, oral) alone where as animals of groups 3, 4 and 5 received ranitidine (50 mg/kg) and plant extract at two doses (250 and 500 mg/kg) along with ethanol. Rats were fasted for 24 h received either plant extract (250 and 500 mg/kg) or control vehicle. After 30 minutes, ulceration was induced by oral administration of absolute ethanol (0.2 mL, oral). The animals were sacrificed after 2 h following administration of ethanol[7]. The stomach was removed, opened along the greater curvature and ulcerated area was calculated. Lesion severity was determined by measuring ulcer index. It was calculated as follows:

$$\text{Ulcer Index} = \frac{10}{x}$$

X is total mucosal area/total ulcerated area.

2.6. Aspirin induced gastric lesions

Group 6 received aspirin (200 mg/kg) alone to induce the gastric ulcer while animals of groups 7, 8 and 9 received (50 mg/kg) and plant extract at two doses (250 and 500 mg/kg) along with aspirin. Aspirin (200 mg/kg×3 days) were administrated once per day to groups of animals for the number of days specified[8]. Animals of the test groups received plant extract suspension orally for 10 days. From day 8 the animals received CMC/plant extract two hours prior to the administration of aspirin. Overnight fasted animals were sacrificed by cervical dislocation one hour after the last dose of aspirin. The stomach was incised along the greater curvature and examined for ulcers.

2.7. Pylorus ligated rats

Stomachs of animals of group 10 were pyloric ligated alone where as animals of groups 11, 12 and 13 received pretreatment of ranitidine (50 mg/kg) and plant extract at two doses (250 and 500 mg/kg) along with pyloric ligation. *F. religiosa* extract (250 and 500 mg/kg) was administrated for a period of 7 days. On day 7, after the last dose of plant extract, the rats were kept for 24 hours fasting. Under light ether anesthesia, the abdomen was opened and pylorus was ligated without causing any damage to its blood vessels. The stomach was replaced carefully and the abdominal wall was closed with interrupted sutures. The animals were deprived of water during post operative period[9]. Four hours after ligation, stomachs were dissected out and lengths of the lesions were measured.

2.8. Biochemical assays

The mucosa of the stomach was scrapped, homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4) and centrifuged at 2 000 g for 10 min at 4 ℃. The supernatant was then kept at −80 ℃ for subsequent measurement of malondialdehyde (MDA) [10], superoxide dismutase(SOD)[11] and catalase enzyme activity(CAT)[12]. Lipid peroxidation (LPO) was determined by thiobarbituric acid (TBA) reaction with MDA, a product formed due to the peroxidation. The absorbance was measured at 532 nm using a Shimadzu UV–160A spectrophotometer (Japan). The protein content was measured in 10 μL supernatant[13] with bovine serum albumin as the standard.

2.9. Statistical analysis

Results are represented as mean±SEM. Statistical difference between the means of the various groups was analyzed using ANOVA followed by Bonferroni's Multiple Comparison Test. Data were considered statistically significant at $P<0.05$.

3. Results

The phytochemical analysis of the hydro alcoholic extract of *F. religiosa* revealed the presence of tannins, sterols, saponins, flavonoids, carbohydrates and proteins. The extract did not produce any toxic symptoms of mortality up to the dose level of 2 000 mg/kg in albino mice and hence the drugs were considered safe for pharmacological study. The results of the present study indicate that the incidence and severity of aspirin, ethanol and pylorus ligation induced ulcerations were significantly reduced by *F. religiosa*(Table1, 2).

4. Discussion

The present study discussed that *F. religiosa* exhibits both gastroprotective and ulcer healing properties. In aspirin, ethanol and pylorus ligation induced gastric ulcer model, the hydroalcoholic extract of *F. religiosa* reduced the ulcer index thus showing the anti ulcerogenic activity. Although there are lots of drugs available in the market for gastric ulcers, including antacids, proton pump inhibitors, anticholinergics and histamine H_2 antagonists are used, most of these drugs produce several adverse reactions, such as gynecomastia, hematopoietic changes, acute interstitial nephritis[14], thrombocytopenia[15], anaphylaxis reactions[16], nephrotoxicity and hepatotoxicity[17]. Therefore medicinal plants with lesser side effects are better alternatives for the treatment of gastric ulcer. Non–steroidal anti inflammatory drugs (NSAIDs) like aspirin induced gastric damage is possibly mediated through leukotrienes production and 5–lipoxygenase pathway[18]. Oral administration of absolute ethanol in rat leads to strong vasoconstriction is accompanied by rapid and vigorous arteriolar dilation and this combination of microvascular events induces damage in mucosal capillaries[19]. The protective effect of the extract of

Table 1

Effect of methanolic extract of *F. religiosa* on different models of ulcer in rats(*n*=6)(Mean±SEM).

Experimental Groups	Ethanol (0.2 mL)	Aspirin (200 mg/kg)	Pylorus ligation
Control	0.05±0.01	0.05±0.01	0.05±0.01
Ethanol/ Aspirin/ Pylorus ligation	4.32±0.22[**]	2.98±0.16[**]	2.08±0.05[**]
Ranitidine (50 mg/kg)	0.77±0.04[**#]	0.53±0.02[*#]	0.41±0.03[**#]
FELD (250 mg/kg)	2.39±0.12[**#†]	1.54±0.14[**#†]	1.05±0.07[**#†]
FEHD (500 mg/kg)	1.03±0.06[**#]	0.66±0.04[**#]	0.53±0.06[**#]

[*]$P<0.05$; [**]$P<0.01$(as compared with untreated group); [#]$P<0.01$(as compared with ulcerogen–treated); [†]$P<0.01$(as compared with ulcerogen + ranitidine–treated).

Table 2

Effect of methanolic extract of *F. religiosa* on lipid peroxidation and antioxidative parameters (*n*=6)(Mean±SEM).

Experimental Groups		MDA (nmol/g wet tissue)	SOD (mU/mg protein)	CAT (mmol/g tissue/min)
Ethanol induced	Control	17.83±1.89	300.50±20.74	36.00±1.84
	Ethanol	78.50±2.23[*]	116.67±3.07[*]	17.83±1.74[*]
	Ranitidine + Ethanol	30.00±2.67[*#]	239.17±9.32[*#]	33.67±1.91[#]
	FELD + Ethanol	51.00±2.11[*#††]	145.33±5.96[††]	21.00±1.51[††]
	FEHD + Ethanol	25.00±1.53[#]	186.50±9.33[*#†]	32.67±1.52[#]
Asprin induced	Control	17.83±1.89	300.50±20.74	36.00±1.84
	Aspirin	62.00±3.49[**]	131.67±5.60[**]	18.33±1.31[**]
	Ranitidine + Aspirin	27.50±1.41[#]	246.17±10.04[*#]	34.33±2.04[#]
	FELD + Aspirin	46.17±3.20[**#†]	156.00±7.64[**†]	22.83±1.80[**†]
	FEHD + Aspirin	25.00±1.53[#]	205.50±6.83[**#]	33.33±1.17[#]
Pyloric ligation induced	Control	17.83±1.89	300.50±20.74	36.00±1.84
	Pyloric Ligation	65.33±3.77[**]	146.83±1.99[**]	20.33±0.71[**]
	Ranitidine + Pyloric Ligation	31.67±2.23[**#]	258.00±5.20[#]	34.17±1.78[#]
	FELD + Pyloric Ligation	47.17±1.30[**#†]	176.67±7.61[**†]	24.17±0.70[**†]
	FEHD + Pyloric Ligation	28.67±2.36[*#]	226.67±6.82[**#]	35.33±0.88[#]

[*]$P<0.01$(as compared with untreated group); [#]$P<0.01$(as compared with ulcerogen–treated); [†]$P<0.05$; [††]$P<0.01$(as compared with ulcerogen + ranitidine–treated).

F. religiosa against the gastric damage may be due to their action against 5–lipoxygenase pathway. The cytoprotective action probably stimulates the prostaglandin synthesis, which in turn protects the gastric mucosa.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Bansal VK, Goyal SK, Goswami DS, Singla S, Rahar S, Kumar S. Herbal approach to peptic ulcer disease: review. *J Biosci Tec* 2009; **1**: 52–8.

[2] Sharma PV. Cikitsastana to Siddhidthana. In: *Caraka Samhita*. 4th ed. Delhi: Chaukhambha Orientalia; 1994, p. 3.

[3] Sharma PV. Treatment of parinamasula. In: *Cakradutta: a treatise on principles and practices of Ayurvedic medicine*. 5th ed. Varanasi: Chaukhambha Orientalia; 1994, p. 249–58.

[4] Rao CV, Sairam K, Goel RK. Effect of mild irritant on gastric mucosal offensive and defensive factors. *Indian J Physiol Pharmacol* 1999; **44**: 185–91.

[5] Shah CS, Quadry JS. *A textbook of pharmacognosy*. 11th ed. New Delhi (India): Shah Prakashan; 1995, p. 333–46.

[6] Dede EB, Dogara FM. The acute toxicological effects of gammalin 20 on the lung and pancreas of guinea pig. *J Applied Sci Environ Manage* 2004; **80**: 33–5.

[7] Agrawal AK, Rao CH, Sairam K, Joshil VK, Goel RK. Effect of *Piper longum* L., *Zingiber officinalis* L. and *Ferula* species on gastric ulceration and secretion in rats. *Indian J Exp Biol* 2000;

38: 994–8.

[8] Rao ChV, Ojha SK, Radhakrishnan K, Govindarajan R, Rastogi S, Mehrotra S, et al. Antiulcer activity of *Utleria salicifolia* rhizome extract. *J Ethanopharmacol* 2004; **91**: 243–9.

[9] Muniappan M, Sundararaj T. Antiinflammatory and antiulcer activities of *Bambusa arundinacea*. *J Ethnopharmacol* 2003; **88**(3): 161–7.

[10] Xu S, Bian R, Chen X. *The pharmacological experimental methods*. Beijing: People′s Health Press; 2005, p. 429–537.

[11] Dandekar SP, Nadkarni GD, Kulkarni VS, Punekar S. Lipid peroxidation and antioxidant enzymes in male infertility. *J Postgraduate Med* 2002; **48**: 186–9.

[12] Yasemin A, Mevhibe BI, Hamdi O, Nazmi O. The mechanism of inhibition of human erythrocyte catalase by azide. *Turk J Biol* 2004; **28**: 65–70.

[13] Mathur SK, Satsangi UK, Jain GC. Effect of antiprostaglandin drugs on human blood–aqueous barrier. *Indian J Opthalmol* 1981; **29**: 97–9.

[14] Ra A, Tobe SW. Acute interstitial nephritis due to pantoprazole. *Ann Pharmacother* 2004; **38**: 41–5.

[15] Zlabek JA, Anderson CG. Lansoprazole–induced thrombocytopenia. *Ann Pharmacother* 2002; **36**: 809–12.

[16] González P, Soriano V, López P, Niveiro E. Anaphylaxis to proton pump inhibitors. *Allergol Immunopathol* 2002; **30**: 342–5.

[17] Fisher AA, Couteur DGL. Nephrotoxicity and hepatotoxicity of histamine H_2 receptor antagonists. *Drug Saf* 2001; **24**:39–42.

[18] Wallace JL. Pathogenesis of NSAID–induced gastroduodenal mucosal injury. *Best Pract Res Clin Gastroenterol* 2001; **15**(5): 691–703.

[19] Ligha AE, Fawehinmi HB. Protection by liquorice in alcohol induced gastric mucosa damage. *Pakistan J Nutr* 2009; **8**(10): 1532–6.