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ANTI-ULCER EFFECT ON THE ETHANOL EXTRACT OF THE BARK & ROOT OF *FICUS RELIGOISA* LINN. IN DIFFERENT EXPERIMENTAL ULCER MODELS IN RATS

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ABSTRACT: *Ficus religiosa L* (Moraceae) is widely distributed in northern part of India has traditionally been used in India as medicinal plant for the treatment of diarrhoea and dysentery. In the present study ethanolic extract of *Ficus religiosa L* was used for investigation of antiulcer activity by using pylorus ligation as antisecretory model and ethanol induced ulceration models as cytoprotective model. Animals pretreated with ethanolic extract of *Ficus religiosa L* at the dose 200 mg/kg and 400 mg/kg showed significant decrease in ulcer index and percentage ulcer protection in all models. The results suggested that the extract at 200 mg/kg and 400 mg/kg showed significant protection ($P<0.001$) by reducing ulcerative lesions when compared with control group of animals. These findings indicate that *Ficus religiosa L.* bark and root extract shows significant antiulcer activity.

INTRODUCTION: Peptic ulcer is the term designated to localized destruction of the inner wall or mucosa of the stomach (gastric ulcer) or the upper part of the small intestine (duodenal ulcer). Peptic ulcers generally occur when there is an imbalance between aggressive gastric factors (acid, pepsin, *Helicobacter pylori* and refluxed bile salts) and defensive mucosal factors [1] (gastric mucosal barrier, bicarbonate secretion, rapid cell turnover, high blood flow). The treatment of peptic ulcer is directed against reduction of aggressive factors or enhancement of mucosal defense of stomach and duodenum with cytoprotective agents.

Endogenous non-protein sulfhydryl (SH) compounds are presumed to participate in gastric mucosal adaptive cytoprotection [2]. For several decades the adage 'NO ACID – NO ULCER' and the drugs used to reduce acid secretion has dominated the pharmacological basis of ulcer therapy. More recently, the role of mucosal factor in peptic ulceration has received much attention and the term "Cyto-protection" was first introduced by Andre Robert in 1979. In general it can be said that there is a plethora of mechanisms of gastric cytoprotection, their relative importance and interdependence being far from clarity. This itself is a point that gastric cytoprotection may be a multifactorial phenomenon.

Ficus religiosa L. (Moraceae), known in India as pimpal, Peepal and Pimpalla is widely distributed in northern part of the India. *Ficus religiosa* is used in traditional medicine for about 50 types of disorders including asthma, diabetes, diarrhea, epilepsy, gastric problems, and inflammatory disorders, infectious and sexual disorders [3].

The juice of its leaves extracted by holding them near the fire can be used as the ear drop. Its power bark has been used to heal the wounds for years. The bark of the tree is useful in inflammations and glandular swelling of the neck. Its root bark is useful for stomatitis, clean ulcers, and promotes granulations. Its roots are also good for gout. The roots are even chewed to prevent gum diseases. Its fruit is laxative which promotes digestion and checks vomiting.

Its ripe fruits are good for the foul taste, thirst and heart diseases. The powered fruit is taken for Asthma. Its seeds have proved useful in urinary troubles. The leaves are used to treat constipation.

Aqueous extract of *Ficus religiosa* release oxidative stress in experimentally induced type 2 diabetes [4]. Stem bark of *ficus religiosa* are reported as anti-inflammatory and analgesic activity [5]. Leaves of *Ficus religiosa* have shown wound healing properties [6]. Although *Ficus religiosa* has been studied to extensively. Hence the present study is an attempt to study antiulcer activity of *Ficus religiosa L*.

MATERIALS AND METHODS

Plant Material: Bark and root of *Ficus religiosa L.* was collected from junnar (Pune) during September. It was authenticated by B.J Medical College ale, Junnar, Pune, India.

Preparation of the extract: The air-dried bark and root of *Ficus religiosa L* was reduced to fine powder (40 size mesh) and around 400 gm of powder was subjected to successive hot continuous extraction (Soxhlet) extraction with ethanol. After the effective extraction, solvent were concentrated using rotary flash evaporator and water was removed by freeze drying and the extract obtained with each solvent was weighed. The extractive value was calculated as % w/w yield and was found to be 6.45%.

Animal used: Healthy adult albino rats of Wistar strain weighing 180-250g were used for this study. Animals were housed at temperature of 24±2 ^{o}C and relative humidity of 30-70%. A12:12 light: day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pelleted rat chaw. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics committee (1409 / a / 11/ CPCSEA) of VIPER.

Phytochemical Evaluation [7]: The ethanolic extract obtained above was subjected to qualitative analytical test for the detection of various chemical constituents viz. Steroides, carbohydrates, glycosides, tannins, proteins, saponin and flavonoids.

Pharmacological Evaluation:

1. **Antisecretory Evaluation:**

a) **Pyloric ligation-induced ulcers** [8]: Four groups of albino Wistar male rats (n=6) were selected. In this model, Group 1 served as normal control (vehicle) received 0.5% Carboxymethyl cellulose (CMC), p. o., and group 2 Ranitidine (50mg/kg, p.o.), whereas groups 2 and 4 animals received ethanolic extract of *Ficus religiosa* (200 and 400 mg/kg,p.o. respectively) daily for 3 days. Animals were fasted overnight prior to start of the experiment, and were given water ad libitum. Pyloric ligation was applied by ligating the pyloric end of the stomach of rats on 3rd day under phenobarbital anaesthesia a dose of 35mg/kg b.w., after 30 min of ethanolic extract or Ranitidine treatment.

Animals were allowed to recover and stabilize in individual cage and were deprived of water during post operative method, after 4 h of surgery. Rats were sacrificed with over dose of ether, stomach was removed and gastric juice was collected for performing gastric secretion study and ulcer scoring was done in stomach as described in the method of Suzuki *et al*.[9] The gastric juice that was collected and centrifuged. The volume and pH was recorded and subjected to bio-chemical estimations like free acidity and total acidity.

TABLE 1: ULCER SCORE

Sr.no.	Stomach colour	Ulcer score
1	Normal colour	0
2	Red colour	0.5
3	Red spot	1
4	Hemorrhagic streaks	2
5	3 > 5 Ulcer	2.5
6	5 > Ulcer	3

The ulcer score was divided by a factor of 10 to get the ulcer index. % ulcer protection was calculated using Ulcer index values.

Ulcer index =

$$\frac{\text{Ulcer index in control} - \text{Ulcer index in Test}}{\text{Ulcer index in control}} \times 100$$

Study of Gastric fluid: The various biochemical parameters like secretions viz. gastric volume, pH, free and total acidity were evaluated.

Gastric Volume: This was measured after centrifuging the gastric fluid, it was allowed to stand, decanted, and poured into the measuring cylinder of graduation 0.01 ml.

Determination of pH: The pH of gastric juice was measuring using the PH meter (Cyber Scan, India**)**

Determination of Free Acidity and Total acidity [10]: 1 ml of gastric juice was pipetted into a 100 ml conical flask. Two to three drops of Topfer's reagent were added and titrated with 0.01N sodium hydroxide (NaOH) which was previously standardized with 0.01 N of oxalic acid. until all traces of the red colour disappears and the colour of the solution was yellowish orange. The volume of alkali was noted. This volume corresponds to free acid. Then 2 or 3 drops of phenolphthalein solution was added and titration was continued until a definite red tinge reappeared. Again the total volume of alkali added was noted. This volume corresponds to total acidity.

Acidity was calculated by using the formula,

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Actual Normality of NaoH} \times 100}{0.1} \text{ meq /1t /100g}$$

Cytoprotective Model:

a) **Ethanol-induced ulcer** [11]: After 12 h of fasting, albino Wistar male rats were divided into four groups of six animals each. Group 1 served as normal control (vehicle) and received 0.5% Carboxy methyl cellulose (CMC), and the group 2 was treated with Ranitidine (50mg/kg). The groups 3 and 4 received 200 and 400 mg/kg of *Ficus religiosa* ethanolic extract respectively. All are administered orally. One hour after treatment, all rats received ethanol (1ml/200gm/kg., body weight) to induce gastric ulcer. After 4 h the animals were sacrificed by cervical dislocation, the stomachs were removed and opened along the greater curvature. Stomachs were gently rinsed with water to remove gastric contents and the mean ulcer index was calculated as described earlier [9].

Statistical Analysis: Results were expressed as mean ± SEM. Statistical significance was determined by one way analysis of variance (one way ANOVA) followed by Dunnet's 't' test with level of significance set at $P < 0.05$.

RESULTS: Phytochemical evaluation of *Ficus religiosa L.* bark and root showed the presence of carbohydrate, glycoside, proteins, volatile oils and tannins.

TABLE 1: EFFECT OF *FICUS RELIGIOSA L* BARK & ROOT ON PYLORUS LIGATED RAT MODEL INDICATING ULCER INDEX & PERCENTAGE ULCER PROTECTION.

Sr. no.	Treatment	Ulcer index	Percentage Ulcer Protection
1	Solvent control (0.5% CMC) 1 ml/kg	58.91±0.90	-
2	Ranitidine (50mg/kg)	9.78±0.51**	83.39
3	*Ficus religiosa* (200mg/kg)	14.35±0.60**	75.64
4	*Ficus religiosa* (400mg/kg)	12.21±0.47**	79.27

• Above value are mean ±SEM; No. of animal in each group=6; * P- value < 0.05, ** p value <0.01 compared with the corresponding control.

The results of effect of ethanolic extract of *Ficus religiosa L.* in Pylorus ligation model was shown in table-1. It indicated that extract at the dose levels of 200 mg/kg and 400 mg/kg produced a significant decrease in the ulcer index, which was also evidenced by significant increase in percentage protection from ulcers at both the dose levels (75.64% & 79.29%) respectively. The activity was comparable and equipotent with that of standard drug Ranitidine (83.39%).

Table 2 shows the results of gastric volume determination of *Ficus religiosa L.* extract treated groups. It indicated that there was a significant decrease in the volume of the gastric juice. The activity was comparable and equipotent to that of Ranitidine ($P<0.01$). The results of gastric pH determination of *Ficus religiosa L.* extract (table 2) treated groups indicated that there was a significant increase in the pH of the gastric juice.The activity was comparable and equipotent to that of Ranitidine ($P<0.01$).

TABLE 2: EFFECT OF *FICUS RELIGIOSA L BARK* & ROOT ON PYLORUS LIGATED RAT MODEL INDICATING PH, GASTRIC VOLUME, FREE ACIDITY AND TOTAL ACIDITY OF GASTRIC JUICE.

Sr. no.	Treatment	Gastric volume (ml)	pH	Free acidity (meq/1/100g)	Total acidity (meq/1/100g)
1	Solvent control (0.5% CMC) 1 ml/kg	6.85±0.26	3.90±0.07	74.15±1.06	132.62±1.05
2	Ranitidine (50mg/kg)	4.63± 0.28**	4.17±0.19**	43.48±1.18**	98.17±1.05**
3	*Ficus religiosa* (200mg/kg)	3.71±0.26**	3.53±0.20*	48.97±1.34**	93.70±1.12**
4	*Ficus religiosa* (400mg/kg)	2.64± 0.24**	4.19±0.28**	41.46±1.05**	88.21±1.15**

Values are mean± SEM, No. of animal in each group= 6; * P value< 0.05, **P value < 0.01 compared with the corresponding control.

The results of free acidity and total acidity estimation of gastric juice of *Ficus religiosa L.* (table 2) treated groups indicated that there was a significant decrease in the free acidity and total acidity of the gastric juice when compared to control animals.

The effect of *Ficus religiosa l.* extract on ethanol induced ulceration model was shown in **table 3**. The results showed that the tested extracts have protective activity for gastric mucosa, since at doses, 200 and 400 mg/kg of extracts. The results of ethanol induced ulceration model suggested that *Ficus religiosa L.* extract at the dose levels of 200 mg/kg and 400 mg/kg produced a significant decrease in the ulcer index (P<0.01), which was also evidenced by significant increase in percentage protection from ulcers at the dose of 200 mg/kg and 400 mg/kg (76.45% &78.46%) respectively. The activity at both the dose levels was comparable and equipotent to that of Ranitidine treated group (P<0.01).

TABLE 3: EFFECT OF *FICUS RELIGIOSA L* BARK & ROOT ON ETHANOL-INDUCED ULCER MODEL INDICATING ULCER INDEX AND PERCENTAGE ULCER PROTECTION.

Sr. no.	Treatment	Ulcer index	Percentage Ulcer Protection
1	Solvent control (0.5% CMC) 1 ml/kg	91.59 ± 0.74	-
2	Ranitidine (50mg/kg)	17.79± 0.61**	80.71
3	*Ficus religiosa* (200mg/kg)	22.84± 0.86**	76.45
4	*Ficus religiosa* (400mg/kg)	19.78±0.43**	78.46

• Above value are mean ±SEM; No. of animal in each group=6; * P- value < 0.05, ** p value <0.01 compared with the corresponding control.

DISCUSSION: *Ficus religiosa* L. ethanolic exaract showed significant dose dependent ulcer protective effect against Pylorus ligation induced ulcers and Ethanol induced ulcers it was comparable to the standard drug Ranitidine.

Moreover, gastric acid is an important factor for the genesis of ulceration in pylorus-ligated rats. The activation of the vagus-vagal reflux by stimulation of pressure receptors in the antral gastric mucosa in the hyper secretion model of pylorus ligature is believed to increase gastric acid secretion [12].

The current data clearly demonstrated that, ethanolic extract of *Ficus religiosa L*. dose dependently decreased the gastric acid and pepsin secretion which showed antisecretory activity of *Ficus religiosa L.*

The antiulcer activity of ethanolic extract of *Ficus religiosa L.* was detected in absolute ethanol-lesions in rats. These models evaluate the drug's capacity to protect the gastric mucosa, differentiating only the severity of gastric lesions. Ethanol-induced gastric damage may be due to stasis in gastric blood flow, which contributes to the development of the hemorrhagic and necrotic aspects of tissue injury.

In addition, ethanols also induces solubilisation of the mucus constituents, decrease the difference of potentional in mucosa thus increasing the flow of Na^+ and K^+ to the lumen and pepsin secretion, and also increases H^+ ions and histamine [13]. The results showed that the extract had an important protective activity for gastric mucosa, since at doses 200 and 400 mg/kg of extracts, they were effective in reducing ulcer lesions in the ethanol model.

CONCLUSION: In conclusion, the oral administration of the ethanolic extract of *Ficus religiosa l.* exhibits anti-ulcer activity in experimental ulcer models. The probable mechanism for its activity may be due to anti-secretary and cytoprotective properties.

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