

## EXPERIMENTAL EVALUATION OF *FICUS RECEMOSA* LINN. FRUITS EXTRACT ON GASTRIC ULCERATION

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### **ABSTRACT**

The ethanolic extract of *Ficus recemosa* was evaluated for its anti-ulcer activity against pyloric ligation, aspirin and ethanol induced gastric ulcer in rats. The fruits extract and standard (Ranitidine, orally at a dose of 50mg/kg body weight) was administered orally at the dose of 100,200,400mg/kg body weight. It was found that *Ficus recemosa* fruits extract showed a dose dependent reduction in the incidence of ulcers in all the three models.

**Keywords:** *Ficus recemosa* fruits, ethanolic extract, ulcer models.

### **INTRODUCTION**

Gastric ulcer, one of the most widespread, is believed to be due to an imbalance between aggressive and protective factors<sup>1</sup>. The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (*Helicobacter pylori*) and drugs<sup>2</sup>. These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility<sup>3</sup>. Drug treatment of peptic ulcers is targeted at either counteracting aggressive factors (acid, pepsin, active oxidants, platelet aggravating factor "PAF", leukotrienes, endothelins, bile or exogenous factors including NSAIDs) or stimulating the mucosal defenses(mucus, bicarbonate, normal blood flow, prostaglandins(PG), nitric oxide)<sup>4</sup>. The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence. Currently there is no cost-effective treatment that meets all these goals. Hence, efforts are on to find a suitable treatment from natural product.

*Ficus racemosa* Linn. belonging to Family Moraceae. A medium-sized to large evergreen or occasionally deciduous tree up to 18 m tall, often with an irregular, shabby crown. Found throughout India to an elevation of about 2000 m in evergreen forests and along the banks of streams in moist ravines in deciduous forests. Also found in Srilanka, Pakistan, Southern China to New Guinea and Northern Queensland, Australia.

The milky juice (latex) from the plant is reportedly used for treating piles and diarrhea, and externally for healing of wounds. Fruits are used as a stomachic & carminative to relieve dysentery, diarrhoea & for the treatment of diabetes. Roots are used in cases of dysentery & diabetes. Barks are used in cases of dysentery. In Mumbai the sap of this plant is a popular remedy for mumps & other inflammatory enlargement<sup>5,6</sup>.

However there are no reports on the antiulcer activity of the plant hence the present study was designed to verify the claims of the native practitioners.

### **MATERIALS AND METHODS**

#### **Collection and Preparation of Extract**

The plant materials used in this study were unripe fruits of *Ficus racemosa* Linn (Family - Moraceae) collected from the botanical garden of NBRI, Lucknow. The plant was identified and taxonomically authenticated by Dr. Sayyada Khatoon by comparison with the herbarium. The voucher specimen (NBP 2240) was lodged in the departmental herbarium of National Botanical Research Institute, Lucknow, India.

The unripe fruits (*Ficus racemosa* L.) (3 kg) chopped in to small pieces and dried under shade/ tray drier in controlled conditions & powdered coarsely. Air-dried powdered fruits of *Ficus racemosa* Linn (1000g) was first defatted with pet-ether twice and then exhaustively extracted with 10 volumes of 50% ethanol for three days. The extract was separated by filtration and concentrated on rotavapour (Buchi, USA) and then dried in lyophilizer (Labconco, USA) under reduced pressure to obtain 78.0 g of solid residue (yield 7.8% w/w)

#### **Preliminary Phytochemical Studies**

The ethanolic extract obtained was subjected to the preliminary phytochemical studies for the presence of Alkaloids, Carbohydrate, flavonoids, glycosides, proteins and free amino acids, gums and mucilages, saponins, sterols, fixed oils, phenolic compounds and tannins using standard methods<sup>7</sup>.

#### **Animals Used**

Wistar rats (150 - 180 g) and albino mice (15 – 18 g) were procured from the central animal house of Central Drug Research Institute Lucknow, India. These were kept in the departmental animal house at 26 ± 2°C and relative humidity 44 –56 %, light and dark cycles of 10 and 14 h



respectively for 1 week before and during the experiment for acclimatization. The animals were provided with standard rodent pellet diet (Dayal, India) and the food was withdrawn 18-24 h before the experiment, though water was allowed *ad libitum*. The laboratory animals are promulgated by the Institutional Animal Ethics Committee, CPCSEA, India (Regd No. 222/2000/CPSEA). All experiments were performed in the morning according to current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals. The standard orogastric cannula was used for oral drug administration in experimental animals.

### **Acute toxicity studies**

The adult male albino mice were selected for acute toxicity study. Before the actual LD<sub>50</sub> determination, a pilot study was made on a small group of mice mainly to select the dose ranges for the subsequent study. The unripe fruit extract of *Ficus racemosa* were taken at various doses levels (100, 200, 400, 800, 1000, 2000 mg/kg body weight) dissolved in 1 % carboxymethyl cellulose administered 10 ml/kg b.w orally to pairs of mice per dose level. The control animals received 1 % carboxymethyl cellulose in distilled water (10 ml/kg) orally. The animals were observed continuously for two hour and then occasionally for further four hours and finally any mortality, Behavior (gross behavior, general motor activity, writhing, convulsion, response to tail pinching, pupil size, fecal output, water intake, feeding behavior, sedation etc.) of the animals and any other toxic symptoms also observed for 72 hours and the animals were kept under observation up to 14 days (OECD, 423)<sup>8</sup>.

### **Statistical evaluation**

Data are expressed as Mean  $\pm$  SEM (standard error of mean) for six rats. The difference among means has been analyzed by unpaired student's t-test.

### **EXPERIMENTAL METHODS**

The Albino rats of Wistar strain are divided into five groups of six animals each. Animals were fasted 24 hours. I group received normal saline 2ml/kg body weight (Control), group II, III, and IV were received extract of *Ficus racemosa* fruits in the dose of 100,200,400mg/kg body weight respectively by oral route. While the V group received Ranitidine 50mg/kg body weight by oral route.

### **Pyloric ligation method**

In this method extracts were administered 30 minutes prior to pyloric ligation, Animals were scarified 4 hour later and the stomach was opened and scoring was done.

### **Aspirin induced ulcer model**

In this method the treated groups received extracts in the dose of 100,200,400mg/kg body weight respectively for 8 days p.o. After 8 days of treatment, the animals were fasted for 24 hours. Ulcer was produced by

administration of aspirin (a dose of 200 mg/ kg orally) on the day of sacrifice. The animals were sacrificed 4 hour later and stomach was then opened to calculate the ulcer index<sup>9,10</sup>.

### **Ethanol induced ulcer model**

The ulcer was induced by administering ethanol. All the animals were fasted for 36 hours before administration of ethanol. The gastric ulcers were induced in rats by administrating absolute ethanol (90%) (1ml/200g) Orally, after 45 min of extracts and standard drug treatment protocol. They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized 1h latter with anaesthetic ether and stomach was incised along the greater curvature and ulceration was scored<sup>11, 12, 13</sup>.

Scoring of ulcer was made as follows

Normal stomach.....	(0)
Red coloration.....	(0.5)
Spot ulcer.....	(1)
Hemorrhagic streak...	(1.5)
Ulcers.....	(2)
Perforation.....	(3)

Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer protection was determined as follows

$$\% \text{ Protection} = \frac{\text{Control mean ulcer index} - \text{Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

## **RESULTS**

### **Phytochemical screening**

The results of preliminary phytochemical screening of the *Ficus racemosa* fruits extracts revealed the presence of alkaloids, flavonoids, carbohydrates, glycosides, tannins, terpenoids, Phenols and absence of fixed oils and steroids.

### **Acute toxicity study (LD<sub>50</sub>):**

No mortality and behavioral changes was found up to the dose of 2000 mg/kg body weight, therefore the dose selected for the antiulcer activity was 100,200,400mg/kg body weight.

### **Anti ulcer study**

A dose-response antiulcer study was done using 100, 200 and 400 mg/kg of ethanolic extract of *Ficus racemosa* Linn. Against various validated gastric ulcer models like Pylorus ligation, aspirin and ethanol induced gastric ulcer. The ethanolic extract was administered orally to various groups. Ulcer were scored and analyzed as described earlier. The result indicated a dose- dependent anti-ulcerogenic activity in ethanolic extract of *Ficus racemosa* Linn. (Tables 1, 2 and 3). The optimal effect was observed at the dose of 400 mg/kg body weight.



**Table-1: Effect of Ficus racemosa fruits extract on pyloric ligation induced gastric ulcer model.**

Group	Treatment	Dose (mg/kg)	Ulcer index (mm <sup>2</sup> /rat)	% protection
I	Control	-	37.3 ± 2.5	-
II	Ficus racemosa extract	100	29.2 ± 2.2 <sup>a</sup>	35.82
III	Ficus racemosa extract	200	22.2 ± 2.0 <sup>b</sup>	56.23
IV	Ficus racemosa extract	400	12.2 ± 1.6 <sup>c</sup>	77.73
V	Ranitidine	50	5.5 ± 1.2 <sup>c</sup>	84.66

Values are mean ± SEM for 6 rats.

<sup>a</sup>P < 0.05, compared to respective control group.

<sup>b</sup>P < 0.01 compared to respective control group.

<sup>c</sup>P < 0.001 compared to respective control group.

**Table-2: Effect of Ficus racemosa fruits extract on aspirin induced gastric ulcers model.**

Group	Treatment	Dose (mg/kg)	Ulcer index (mm <sup>2</sup> /rat)	% Protection
I	Control	-	15.6 ± 3.9	-
II	Ficus racemosa extract	100	1.2 ± 1.7	2.0
III	Ficus racemosa extract	200	2.2 ± 1.6 <sup>a</sup>	6.07
IV	Ficus racemosa extract	400	6.3 ± 2.4 <sup>a</sup>	12.04
V	Ranitidine	50	10.7 ± 2.8 <sup>b</sup>	31.56

Values are mean ± SEM for 6 rats.

<sup>a</sup>P < 0.05, compared to respective control group.

<sup>b</sup>P < 0.01 compared to respective control group.

**Table-3: Effect of Ficus racemosa fruits extract on Ethanol acid gastric ulcer model.**

Group	Treatment	Dose (mg/kg)	Ulcer index (mm <sup>2</sup> /rat)	% Protection
I	Control	-	22.6 ± 3.9	-
II	Ficus racemosa extract	100	14.7 ± 2.8	41.56
III	Ficus racemosa extract	200	12.3 ± 2.4 <sup>a</sup>	62.04
IV	Ficus racemosa extract	400	8.2 ± 1.6 <sup>b</sup>	80.72
V	Ranitidine	50	5.4 ± 1.7 <sup>b</sup>	89.51

Values are mean ± SEM for 6 rats.

<sup>a</sup>P < 0.05, compared to respective control group.

<sup>b</sup>P < 0.01 compared to respective control group.

## DISCUSSION AND CONCLUSION

The etiology of peptic ulcer is unknown in most of the cases, it is generally accepted that gastric ulcers results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through endogenous defense mechanisms <sup>14</sup>. The excess gastric mucus formation by prostaglandins (PG) includes both increases in mucosal resistance as well as a decrease in aggressive factors, mainly acid and pepsin <sup>15</sup>. Inhibition of PG synthesis by aspirin coincides with the earlier stages of damage to the cell membrane of mucosa, parietal and endothelial cells <sup>16</sup>.

Pylorus ligation induced ulcer was used to study the effect of fruit extracts on gastric acid secretion and mucus secretion. The ligation of the pyloric end of the stomach

causes accumulation of gastric acid in the stomach. This increase in the gastric acid secretion causes ulcers in the stomach.

Ethanol induced gastric ulcer was employed to study the cytoprotective effect of the extracts. Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the haemorrhage and necrotic aspects of tissue injury. Alcohol rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intra cellular membrane permeability to sodium and water. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium <sup>17, 18</sup>.



The plant reported to be anti-ulcer in traditional system of medicine and in the present research programme the plant was found to have the significant anti-ulcerative activity in rats. The preliminary phytochemical studies revealed the presence of flavanoids in fruits extract of *Ficus racemosa* in major concentration. Various flavanoids have been reported for its anti-ulcerogenic activity with good level of gastric protection<sup>19</sup>. So the possible mechanism of anti-ulcer action of *Ficus racemosa* fruits may be due to its flavanoids content. In this study we observed that *Ficus racemosa* provides significant anti-ulcer and cytoprotective effect against gastric ulcers in rats. Further study need to be carried out regarding exact mechanism, isolation and characterization of active principal responsible for anti-ulcer activity.

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